THE NUTRITIVE VALUE OF LACTOSE

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ONE FIGURE

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The fact that lactose passes at least in part unabsorbed to the large bowel, raises the question of its nutritive value. Textbooks frequently state that lactase is present during infancy, but disappears, particularly in man, during later life. Evidence on this latter point, however, to our knowledge is very meager. That lactose passes through to the large bowel is demonstrated by its action on the bacterial flora (Rettger and Cheplin, '21; Mitchell, '27), lactic acid production and its effect on the pH of the colon contents (Robinson and Duncan, '31; Kline et al., '32), and the apparently secondary effect on calcium and phosphorus assimilation (Kline et al., '32; Bergeim, '26; Robinson et al., '29). It seems logical to assume that the nutritive value of lactose is inversely proportioned to its bacterial utilization and fermentation in the colon unless some of the intermediary products of fermentation, such as lactic acid, are reabsorbed from the colon and used as fuel in the body.

The fact that lactose cannot be used as such by the animal organism was first shown by Voit (1897) in demonstrating that lactose administered subcutaneously was practically completely eliminated in the urine. The fact that lactose was also poorly used when orally administered as far as glycogen formation in the dog was concerned was first shown by Murschhauser ('11). He found that 50 gm. of sugar in 8

hours produced only a value of 6 for lactose as compared to 100 for glucose. These findings were consistent with those of Lusk ('15) that lactose in the dog failed to produce the specific dynamic effect shown by sucrose or glucose and failed to materially increase the respiratory quotient. Lusk concluded that lactose was not oxidized at all and that this was due to the absence of lactase in the intestine. In the phlorhizinized dog, however, Deuel and Chambers ('25) found that glucose could be formed, at least in part, from lactose. In their experiments there was a delay in the elimination of the 'extra sugar' after lactose ingestion as compared with glucose, fructose and galactose. Only 50 per cent of the ingested lactose was excreted as glucose, while that for the other sugars was practically 100 per cent.

The rat, on the other hand, appears to be more capable of utilizing lactose. Greisheimer and Johnson ('30) showed that, after feeding rats for 16 to 18 days on diets containing 87.5 per cent of the total caloric value in sugar, the liver glycogen (3.15 per cent) on the lactose diet was only 62 per cent of the liver glycogen (4.89 per cent) on glucose or sucrose diets. Mitchell ('27) studied the growth of the rat on diets containing from 30 to 60 per cent carbohydrate and found growth on 30 per cent lactose was only 50 per cent of that on the other carbohydrates. She noted that the per cent of food sugar lost in the feces on diets as high as 60 per cent carbohydrate was only 3.12 per cent greater for lactose than for sucrose. These experiments, however, did not answer the question of the nutritive value of lactose, inasmuch as the total amount of food eaten was not reported. Furthermore, the growing animal does not lend itself to this type of experiment, inasmuch as it has been shown that lactose may affect the rate of skeletal growth (Kline et al., '32).

PLAN OF EXPERIMENTS

Young full-grown rats were fattened on a calf meal diet[1] and then put in small individual cages and placed on daily

[1] 'G. L. F. calf meal' P-18, F-4, C-56, indigestible 22.

weighed diets approximately 30 to 40 per cent below maintenance for each rat. They were weighed every third day and in the course of 20 to 40 days lost from 40 to 50 gm. body weight. After this weight loss, lactose, glucose or sucrose was added to the diet in an amount represented by the difference between the maintenance and sub-maintenance reducing diet. Thus, if 100 parts of meal represented the maintenance diet and 65 the reducing diet, the amount of sugar added was 35. Each rat was given a daily weighed portion of the meal-sugar mixture equivalent to 100 parts instead of 65 of the meal alone on the reducing diet. In this manner, the protein, fat and other dietary constituents were unchanged during the reducing and weight-gaining periods, the only difference being the sugar added during the second period of the experiments.

Since the caloric intake of the rats was constant during the periods of lactose, glucose and sucrose feeding, any difference in weight curves should be due to variation in the extent of assimilation of the sugars unless there was a concomitant change in total activity of the animals. To control this latter factor a separate set of experiments was carried out to study the voluntary activity of the rats on the glucose, lactose and sucrose diets. A group of ten rats was placed individually in the conventional revolving cages of a circumference of 1 meter. They were given daily 15 gm. of a diet consisting of 65 parts meal and 35 parts sugar. The food not consumed was weighed and the daily food consumption calculated.

RESULTS

The results of the weight gaining experiments are graphically shown in figure 1. Curve 1 shows the average weights of nine rats during the latter third of the weight losing period and during the period when glucose was added. Curve 2 shows the comparable results in the averages of ten rats when the equivalent amount of lactose was substituted. After 32 days on the glucose diet the gain in weight was 35 gm., while on the lactose diet the gain was only 22.5 gm. the equivalent of only 64 per cent of the former.

In curves 3 and 4 are shown the comparable average weight gains of groups of rats of ten each on sucrose and lactose diets. In general, the glucose and sucrose curves are approximately alike. In 21 days the weight gain of the rats on the sucrose diet was 33 gm., while on the lactose diet the gain was only 20 gm.—representing only 61 per cent of the sucrose gain. In the group of rats on the lactose diet represented by

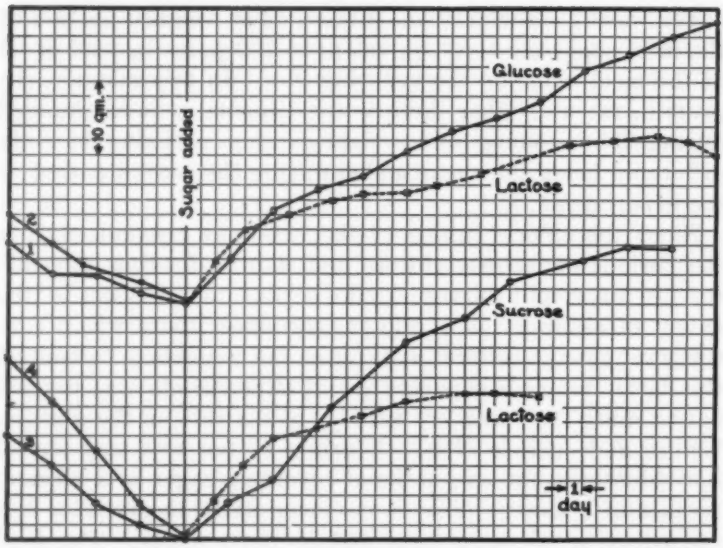

Figure 1

curve 4, the experiment was terminated at the end of 24 days, because of the refusal of the rats to eat all the diet. At no time was a diarrhea noted while on the high carbohydrate diets. Only traces of fermentable reducing substances were found in the feces after acid hydrolysis and protein removal during the periods of lactose feeding. On her 60 per cent lactose diets, which was a somewhat higher percentage of sugar than we used, Mitchell found a daily excretion per rat of 172 mg. per cent reducing substances expressed as sugar.

Since the acid hydrolysis of feces produces non-fermentable reducing substances, the values found by Mitchell and expressed as sugar are probably too high.

That the relative decrease in the weight gain on lactose as compared with glucose or sucrose was not due to increased voluntary activity is shown in table 1. Young rats nearly full grown were used, inasmuch as it is difficult to train full grown rats to run in the exercise cages. Meal was fed during the

TABLE 1

The effect of lactose feeding on the voluntary activity of nine rats

DATE, 1933	WEIGHT (AVERAGE), GRAMS	FOOD CONSUMED PER DAY (AVERAGE), GRAMS	REVOLUTIONS PER DAY (AVERAGE)
Meal only			
7–3 to 7–18			100
Meal 65, glucose 35			
7–18 to 7–21	206	18.3	95
7–21 to 7–25	221	15.7	120
7–25 to 7–30	221	13.9	120
7–30 to 8–5	220	13.9	128
8–5 to 8–11	224	15.1	122
8–11 to 8–17	220	14.0	125
Average	219	15.1	118
Meal 65, lactose 35			
8–17 to 8–18	218	12.6	122
8–18 to 8–25	223	13.3	105
8–25 to 9–1	209	13.2	95
9–1 to 9–8	208	13.2	97
Average	214	13.1	105

first period of 2 weeks until the rats reached a level of uniform activity. Following this the rats were placed on a 26-day period of glucose meal which was succeeded by a 20-day period of lactose meal.

During the lactose period there was at first a gain and then a progressive drop in weight in the averages of the nine rats. This initial relative gain and subsequent loss in weight on lactose as compared with glucose and sucrose is also indicated in the groups shown in figure 1. Both average food

intake and voluntary activity decreased on the lactose period as compared with the glucose period. These experiments showed quite conclusively that the relative weight loss or diminished weight gain on the lactose diets was not due to increased voluntary activity.

The possibility that hydration changes may have entered into the differences between lactose and glucose or sucrose weight gain has not been ruled out in these experiments. Since the respective differences in weight were 36 and 39 per cent, it is improbable that there was this degree of dehydration on the lactose diets.

SUMMARY

The nutritive value of lactose in the rat as compared with that of glucose and sucrose was studied by noting the weight gain after the sugars were added in equivalent amounts to a sub-maintenance diet.

The weight gain after lactose was added to the diet was at first slightly greater than that after glucose or sucrose addition, but after the fifth to the ninth day the weight gain on the lactose diet was distinctly less than that for the other two sugars. After 32 days, it was only 64 per cent of the gain on glucose and after 21 days, only 61 per cent of the gain on the sucrose diet.

On the lactose diets the weight gain curves had a tendency to flatten out considerably before those for glucose or sucrose. The level of the flattening of the lactose curve was 50 per cent below that of the sucrose curve.

The voluntary activity of rats on a lactose diet was not greater than that of a group on a glucose diet. Activity, therefore, is not a factor in the poorer nutritive value of lactose.

A considerable portion of ingested lactose, approximately from 40 to 50 per cent, may be lost to the rat as far as weight or energy relationships are concerned.

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IRRADIATED VITAMIN B COMPLEX AND DERMATITIS

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ONE FIGURE

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Hogan and Hunter ('28) observed that one member of the vitamin B complex is destroyed by ultraviolet irradiation. The original procedure was modified by Hogan and Richardson ('32) and they observed that rats which receive irradiated dried yeast develop a severe dermatitis. In our experience the incidence of dermatitis and the percentage of mortalities have both been practically 100 per cent. After this degree of success had been attained, it seemed desirable to reexamine the technic in an effort to effect additional improvements and to eliminate any precautions that might prove unnecessary. The purpose of this paper is to describe the technic now in use, though the earlier publications should be consulted for complete details.

EXPERIMENTAL

Albino rats are used as experimental animals. The litters we expect to use, with their mothers, are transferred to floors of metal screens, without bedding, usually at 15 days of age. If a litter is under the normal weight this transfer may be delayed 1 or 2 days. From this time on the mother and her young are supplied with the experimental diet, which is free

[1] Contribution from the Departments of Animal Husbandry and Agricultural Chemistry, Missouri Agricultural Experiment Station. Journal series no. 376.

of the antidermatitic factor. The litters are usually weaned at 21 days but if they weigh less than 25 gm. at that time they are left with their mothers until they do attain that weight. Immediately on weaning, the young are transferred to individual cages and the experimental period begins.

The experimental ration formerly used, no. 1545, is made up as follows: Casein, 20; sucrose, 71; cellulose, 3; salt mixture (Osborne and Mendel, '19), 4; cod liver oil, 2. The casein had been thoroughly extracted with acidified water, with alcohol and with ether, and the sucrose had been recrystallized. The food is supplied ad libitum, and no records are taken as to the amount consumed.

The results thus far have been most consistent when dried yeast[2] was the source of vitamin B. During irradiation this is spread out in a thin layer, and stirred thoroughly at least four times. The distance from the arc to the yeast is 14 cm., and irradiation is continued for 10 hours. The yeast supplement for each animal is weighed out in a small glass container and usually it is consumed immediately. Except in special cases, the daily allowance is limited to 100 mg. When receiving this material rats quickly develop severe dermatitis, and the survival periods are short. A photograph of a typical case of dermatitis is reproduced in figure 1.

STUDIES ON THE PROCEDURE

1. Intensity of irradiation. This portion of the technic has not been changed. If the period of irradiation is too short, there will not be sufficient destruction of the antidermatitic factor; and if too prolonged, it is possible that there may be too much destruction of the antineuritic factor.

2. Preliminary preparation. Originally the experimental animals, with their mother, were placed on screens at 5 days of age. At 15 days the mother and litter were given the experimental ration, and the litter was weaned at 21 days. Somewhat later, the transfer of the litter to screens was delayed until the fifteenth day, with no appreciable effect on

[2] Secured from the Harris Laboratories, Tuckahoe, New York.

the results. A study was then made to see if the preliminary preparation could be dispensed with entirely, but it was decided this was not advisable. A delay of 6 days in subjecting the animals to experimental conditions postpones the appearance of dermatitis, and lengthens the survival period. Our observations on this point are summarized in table 1. The vitamin carrier was dried yeast.

3. Purification of the protein. Casein which had been leached with acid water only was substituted for the prepa-

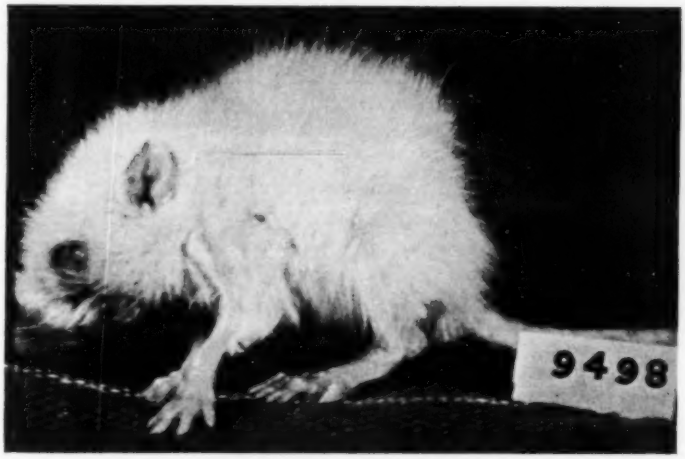

Fig. 1 Characteristic lesions have appeared on the feet, nose, and mouth, but there is no loss of fur elsewhere. The eyelids are adherent.

ration which had also been extracted with alcohol and ether, but more consistent results were obtained when the alcohol and ether extractions had not been omitted.

4. Influence of carbohydrate component. Commercial sucrose has been substituted for the recrystallized preparation. At first there was reason to believe this substitution may have lengthened slightly the survival periods. We are now using commercial sucrose exclusively, and find that for our purpose it is only slightly, or not at all, inferior to the recrystallized preparation. An attempt was also made to use

cornstarch[3] instead of sucrose, but this substitute was abandoned in studies of dermatitis because the lesions did not develop.

It seemed improbable that the protective action of cornstarch could be due to the starch molecule itself, so an attempt was made to extract the active agent with 95 per cent alcohol. Preliminary studies have shown that this extract is very effective in healing dermatitis, though it does not permit the rats to grow. Furthermore, when extracted starch is substituted for the sucrose of ration 1602, healing does not

TABLE 1

Preliminary preparation before weaning reduces length of experimental periods

	NUMBER OF ANIMALS	DERMATITIS		MORTALITY		WEIGHT	
		Average age at onset days[1]	Per cent	Average age at death days[1]	Per cent	Average initial grams	Average final grams
Preliminary preparation[2]	14	54.2 (45–63)	100	67.1 (55–91)	100	26.4	28.4
No preliminary[3] Preparation	18	73.2 (55–98)	94.4[4]	104.8 (62–182)	100	34.2	44.0

[1] Days from birth.

[2] Screens on fifth day; ration 1545 on fifteenth day; weaned, vitamin supplements on twenty-first day.

[3] Weaned, vitamin supplement on twenty-first day. No preliminary preparation.

[4] One died without developing dermatitis, another had only a mild case.

occur. The observation that the starch extract heals dermatitis, but does not enable the rats to resume growth, is interpreted as evidence that more than one member of the vitamin B complex is affected by irradiation.

5. Thirteen per cent of fat was substituted for an equal amount of sucrose, but when this change was made the results were much less consistent. The appearance of dermatitis was delayed and some died without developing lesions. Two per cent of cod liver oil is used in all rations, but at the worst this does not interfere seriously with the development of dermatitis.

[3] Staley's pure food powdered cornstarch, manufactured by the Staley Mfg. Co., Decatur, Illinois.

As a result of our studies on the constituents of the diet ration 1669 is now used almost exclusively. This differs from ration 1545 only in containing commercial sucrose instead of the recrystallized preparation.

Sherman and Derbigny ('32) stated that the type of dermatitis they were investigating developed much more quickly and was much more severe when the ration was low in protein than when it was high. We compared by our technic a ration that contained 30 per cent of casein with one that contained 10 per cent, and our experience was contrary to that of Sherman and Derbigny. Rats receiving the ration with 30 per cent of protein developed dermatitis more quickly, and survived for shorter periods of time than did those on the low protein diet. This is regarded as additional evidence that the factor with which we are concerned is not the vitamin G of Sherman and of other workers in this field.

An attempt was also made to substitute dried egg white and milk albumin for casein. Only a few animals were studied, but either of these proteins may be used. Dermatitis developed more quickly and the survival periods were shorter when dried egg white was employed, but it is possible that this difference would disappear if the milk albumin had been washed more thoroughly. The various rations we have used are described in table 2, and the results obtained are summarized in table 3.

Irradiation of solutions. If the supply of ultraviolet arcs is limited, it is not possible to irradiate sufficient yeast for any considerable number of animals. We have attempted, therefore, to use soluble vitamin B carriers, as it seemed probable that larger quantities could be irradiated effectively in solution than in the dry form. A number of preparations have been studied, but those that seemed most promising were a water extract of yeast and a mixture of equal parts of tikitiki[4] and liver extract.[5] When supplied singly, neither tikitiki nor

[4] Prepared in this laboratory.

[5] We are much indebted to Dr. David Klein, of the Wilson Laboratories, Chicago, Illinois, for generously supplying us with this material.

liver extract is a suitable source of the vitamin B complex. However, the mixture and also the water extract of yeast supported satisfactory growth in controls that received untreated preparations.

When the supplement was irradiated in solution, a volume of 125 cc., containing 15 gm. of solids, was placed in a Pyrex glass baking dish 28 cm. long and 16.5 cm. wide. This dish

TABLE 2

Composition of rations[1]

NUMBER OF RATION	465	1256	1602	1612	1616	1617	1669	1670	1729	1730	1731	1732
Casein 80[2]	20		20	20								
Casein 180[3]		20			20	20	20	20	30	10		
Lactalbumin[4]											20	
Ovalbumin[5]												20
Commercial sucrose			71				71		61	81	71	71
Recrystallized sucrose		58			58	58						
Cornstarch	58			71				71				
Crisco						13						
Lard	10	13										
Milk fat					13							
Salts	4	4	4	4	4	4	4	4	4	4	4	4
Cod liver oil	5	2	2	2	2	2	2	2	2	2	2	2
Cellulose	3	3	3	3	3	3	3	3	3	3	3	3

[1] The vitamin B carrier, whether irradiated or untreated, is supplied separately. When the amount is not specified, it will be understood that the total quantity is 100 mg.

[2] Casein 80, extracted with dilute acid.

[3] Casein 180, extracted with dilute acid, with alcohol, and with ether.

[4] Prepared in this laboratory.

[5] Secured from T. M. Duche & Sons, Inc., New York City.

was 14 cm. beneath the mercury arc, and was enclosed on the sides and bottom with a metal jacket through which a stream of tap water was constantly flowing. This jacket was supported on a mechanical rocker so the vitamin solution was in constant motion. The temperature of this solution was never observed to rise above 35°C. Distilled water was added at intervals to prevent undue concentration. Experience indicated this material should be irradiated for 15 hours.

TABLE 3

Response to irradiated vitamin B carriers is affected by constituents of the diet

RATION NO.	NUMBER OF ANIMALS AND TYPE OF SUPPLEMENT	DERMATITIS		MORTALITY		WEIGHT	
		Average age at onset days	Per cent	Average age at death days	Per cent	Average initial grams	Average final grams
		Casein 80, commercial sucrose					
1602	10—yeast	65.9	100	81.2	100	25.6	33.3
		Casein 180, commercial sucrose					
1669	5—yeast	52.2	100	77.8	100	24.5	32.3
	16—TtL[1]	54.4	100	64.2	100	25.1	31.6
		Casein 180, cornstarch					
1670	8—yeast	....	0	121.6	62.5	22.6	39.8
	4—TtL	....	0	56.0	25	20.2	43.7
		Casein 80, cornstarch, lard					
465	6—yeast	....	0	....	0	36.3	77.6
	4—TtL	....	0	97	25	34.0	61.5
		Casein 80, cornstarch					
1612	7—yeast	....	0	66	14.2	20.8	59.1
		Casein 180, recrystallized sucrose, lard					
1256	5—yeast	63.0	20	95	20	31.4	63.6
	5—TtL	....	0	104	100	20.4	37.0
		Casein 180, recrystallized sucrose, milk fat 13					
1616	5—yeast	65.3	60	88.3	60	31.2	48.0
	3—TtL	....	0	140.3	100	26.3	40.0
		Casein 180, commercial sucrose, crisco					
1617	7—yeast	72.0	42.9	72.5	71.4	30.8	42.7
	5—TtL	....	0	160.5	80.0	25.6	45.2
		Casein 180, 30 per cent, commercial sucrose					
1729	8—TtL	46.7	100	60.8	100	26.3	28.7
		Casein 180, 10 per cent, commercial sucrose					
1730	10—TtL	63.0	50	113.2	100	25.9	30.5
		Lactalbumin, commercial sucrose					
1731	3—TtL	56.0	100	100.6	100	27.6	32.3
		Ovalbumin, commercial sucrose					
1732	3—TtL	49.0	100	68.3	100	26.0	27.6

[1] Contains equal amounts of dry matter from tikitiki and from liver extract.

Some of our observations on this point are reproduced in table 4, section 2. The controls are shown in section 1.

It seemed possible that the destruction of the antidermatitic factor may not be due to the ultraviolet region of the spectrum, so a few animals were given a preparation that had been irradiated through ordinary window glass. These animals made considerable gains in weight and did not develop

TABLE 4

Time necessary to irradiate when supplements are in solution

NUMBER OF ANIMALS	TIME IRRADIATED, HOURS	DERMATITIS		MORTALITY		WEIGHT	
		Average age at onset days	Per cent	Average age at death days	Per cent	Average initial grams	Average final grams
1. Supplement, dried yeast							
Arc A							
9	10	48.7	100.0	65.4	100.0	28.1	29.5
Arc B							
5	20	56.0	100.0	85.2	100.0	33.6	33.4
2. Supplement, TtL [1]							
6	15	57.4	100.0	69.5	100.0	24.6	34.3
3	10	71.0	33.3	82.0	33.3	25.3	61.3
3	5	....	....	51.0	33.3	25.0	62.0
3. Supplement, TtL,[1] irradiated through glass							
3	15	....	....	....	....	21.0	75.6
Irradiated direct							
3	15	51.3	100.0	65.3	100.0	20.0	32.0

[1] Contains equal amounts of dry matter from tikitiki and from liver extract.

dermatitis. A summary of these observations is included in table 4, section 3.

It was concluded that the mixture of tikitiki and liver extract could be used successfully, but it is not regarded as an ideal preparation for our purpose. The survival periods are no longer than on irradiated yeast, but rats receiving this mixture do not develop the most severe type of dermatitis. The water extract of yeast has not yielded consistent results.

The first preparation seemed ideal as, after irradiation, it produced a severe type of dermatitis. When a second preparation was put into use, practically all the animals died in 4 or 5 weeks without developing lesions of any kind. It was thought this concentrate may have been injured in some manner, presumably by overheating, so it was replaced by a third yeast extract. When this new preparation is irradiated it produces severe dermatitis and, up to date, it is the most satisfactory we have used. There is reason to believe the destruction of the antineuritic factor is relatively slight, probably because the irradiation is conducted at a lower temperature. Our data on this new material are not complete, however, and are not included in this paper. A summary of our observations with dried yeast, the mixture of tikitiki and liver extract, and the water extract of yeast follows in table 5. These observations extended from May, 1932, to December, 1933.

TABLE 5

Effect of irradiation on various vitamin B carriers

SOURCE OF VITAMIN	NUMBER OF ANIMALS OBSERVED	NUMBER OF CASES OF DERMATITIS
Dried yeast	198	190
Tikitiki + liver extract	69	69
Water extract of yeast	86	53

It will be observed that eight rats which received irradiated yeast did not develop dermatitis, but presumably this was because death intervened before there was time for the lesions to develop. None of these animals was more than 69 days old when it died. All of the rats that were given the mixture of irradiated tikitiki and liver extract developed lesions of the skin, but in many cases the symptoms were very mild. It may be that the failure to develop acute symptoms was due to partial destruction of the antineuritic factor, which killed the animals before there was time for the dermatitis to become severe. Approximately half of the animals which received this mixture of supplements were diagnosed as hav-

ing severe polyneuritis, and many of the others had mild cases. It should be mentioned that these symptoms were not regarded as typical, for they seemed identical in every way with those described by Miss Reader ('30). She believes this condition is caused by simultaneous deficiency of B_1 and B_4. No abnormalities that would suggest polyneuritis were ever observed in rats that received irradiated yeast.

It also developed that the type of lesion now under discussion is healed by tikitiki, at a daily dosage of 100 mg. of dry matter. However, another type developed some weeks later which is apparently identical with the form described by Sherman and Sandels ('31). Since dermatitis has never been ascribed to a lack of vitamin B, we feel the type we have produced may be due to destruction of the B_4 of Miss Reader ('30).

It is now our custom when testing a vitamin supplement for antidermatitic activity by the prophylactic procedure not to use a depletion period. When the curvative method is used, which we prefer, the animals are deprived of all members of the vitamin B complex for the first 7 to 10 days of the experimental period.

Completeness of destruction due to irradiation. Studies have also been undertaken to determine whether the antidermatitic factor had been completely destroyed by irradiation. A small number of rats was given more than the minimum dosage we employ in routine studies. It developed at once that destruction was not complete, for when the amount of the supplement was greatly increased dermatitis was delayed, or did not appear at all.

Some effort has been made to estimate the degree of destruction of the antidermatitic factor by determining the minimum amount of untreated yeast that will heal dermatitis. To this end a few rats that had developed mild cases of dermatitis were given graded doses of untreated yeast as the sole source of the vitamin B complex. These observations are summarized in table 6.

It does not seem possible to estimate with any precision just what part of the antidermatitic factor is destroyed by irradiation. The rats that received 60 mg. daily of untreated yeast healed decisively, and those that received 50 mg. improved without complete healing.

It is our opinion that 200 mg. of irradiated yeast is at best no more effective in healing dermatitis than is 50 mg. of untreated yeast. It is estimated, therefore, that about 75 per cent of the antidermatitic substance is destroyed by the technic employed. We are now attempting to devise a procedure that will eliminate this substance completely, and still leave an adequate supply of the antineuritic principle.

TABLE 6

Dosage of untreated yeast to heal dermatitis

NUMBER OF RATS	QUANTITY OF YEAST	REMARKS
	mg.	
3	40	None healed
3	50	Improved
2	60	All healed
2	80	All healed

SUMMARY

1. The procedure for producing dermatitis has been slightly modified.

2. Cornstarch contains the factor that prevents dermatitis. The results were less consistent when 13 per cent of fat, hydrogenated cotton seed oil, milk fat, or lard, were included in the ration.

3. For studies of the effect of irradiation on the vitamin B complex, dried yeast is the most suitable carrier that has been examined. Approximately 75 per cent of the antidermatitic activity is destroyed by the technic described.

4. The degree of destruction of the antineuritic factor in dried yeast has not been assayed, but it is not sufficient to interfere with the method.

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SOME EFFECTS OF THE COMPOSITION OF THE DIET ON THE VITAMIN B AND THE VITAMIN G REQUIREMENT OF THE GROWING RAT [1]

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EIGHT FIGURES

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A previous communication from this laboratory (Guerrant and Dutcher, '33) contained data which suggested that the fiber content of the diet might have a marked effect on the amount of vitamin B complex required by the rat. A review of the available literature at that time revealed that other investigators had made similar observations concerning some of the other constituents of the basal diets commonly employed in vitamin B and vitamin G technics. Most of these observations, however, had been made in connection with the protein and fat content of the diet.

Drummond, Crowden and Hill ('22), Reader and Drummond ('25), Hartwell ('25), Reader and Drummond ('26), Hassen and Drummond ('27), Sherman and Gloy ('27), Hartwell ('28) and Guha ('31) had reported observations concerning various relations of the B complex vitamins to protein utilization. The role played by fats in this connection had been studied by Evans and Lepkovsky ('28, '29) and Guha ('31).

It was difficult to account for the variations in the conclusions drawn by the above investigators. It seemed possible,

[1] Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station, March 15, 1934, as technical paper no. 642.

however, that a part, at least, of the lack of uniformity of results might have been due to the fact that vitamin B was not recognized as a complex at the time when some of the experiments were conducted. This fact and, in addition, the wide variations in the composition of basal diets employed by different laboratories in their vitamin B and vitamin G technics, led us to believe that a study of the effects of variations in the composition of the diet on the vitamin B and the vitamin G requirement of the rat could be made with profit.

During the course of this investigation, other communications have been published by Sherman and Derbigny ('32), Evans and Lepkovsky ('32 a, '32 b), Lecoq ('32 a, '32 b, '32 c, '33), Lecoq and Savare ('33), Prunty and Roscoe ('33), Hogan and Pilcher ('33), Francis, Smith and Mendel ('33), Gregory and Drummond ('32), and by Patras and Templeton ('33), which have a bearing on this subject.

The data herein reported give some of the results of a series of experiments carried out during the past 2 years in an effort to gain some insight into the influence of the composition of the diet on the vitamin B and the vitamin G requirement of the growing rat.

EXPERIMENTAL

The general plan followed was to feed groups of rats on diets of varied composition, but deficient in both vitamins B and G until early symptoms of vitamin B deficiency appeared; then to supplement such diets with daily dosages of vitamin B concentrate, while continuing the experiment for several weeks until some manifestations of vitamin G deficiency became apparent, at which time further supplementation was effected by daily additions of autoclaved yeast and the animals were continued under observation for several additional weeks.

Both piebald and albino rats were used in the investigation. These animals were placed on experiment in groups of from 5 to 12 animals each, care being taken in their group distribution in order to minimize both litter and sex variations.

Groups of rats, 20 to 21 days old and weighing 39 to 46 gm. each, were placed and maintained in individual cages provided with raised screen grids, and were fed liberal quantities of the diets under consideration. Clean distilled water was kept before the animals at all times. Small additions of iodine were given at weekly intervals. Weekly records were made of the quantity of food consumed, the change in body weight, and the general appearance of each animal. After a preliminary depletion period of 21 days on such diets, each animal was given a measured daily dosage of the vitamin B concentrate during the next 42 to 56 days as a supplement to the diet. At the end of this period, the diet of each animal was further supplemented by weighed daily allotments of autoclaved yeast and the animal was continued under observation for at least 6 additional weeks. Thus all animals which survived the experimental period had existed for 21 days on a diet deficient in both vitamins B and G, followed by 42 to 56 days on the same diet supplemented by daily allotments of vitamin B, and finally for at least 42 days on this diet supplemented by both vitamins B and G.

The vitamin B supplement used in these studies was prepared from dried brewer's yeast. The dry yeast was extracted by percolation with 95 per cent alcohol which had been acidified with concentrated hydrochloric acid (5 ml. of acid per liter of alcohol) as long as the percolate remained colored. The combined percolate was concentrated under reduced pressure until the residue assumed a sirupy consistency. This concentrate was placed in a separatory funnel, an equal volume of water was added, and the mixture was shaken with three successive portions of ether (100 ml. of ether for each kilogram of yeast extracted) in order to remove most of the fatty materials. After partially neutralizing the excess acid with sodium bicarbonate, the concentrate was again reduced to a sirupy consistency by vacuum distillation, and was maintained at a sub-zero temperature for 24 hours. The insoluble materials were filtered off rapidly by means of suction, and sufficient 95 per cent alcohol was

added to make a volume such that 1 ml. of the concentrate represented 10 gm. of the original yeast. This concentrate was kept in the refrigerator during the course of the investigation. A daily dose of 0.1 ml. of this solution was used as a source of vitamin B. Previous tests had shown this quantity to be sufficient to stimulate appreciable growth, when fed to rats receiving a diet complete in other respects.

The vitamin G supplement was prepared from baker's yeast by moistening and dry yeast with a 10 per cent solution of sodium bicarbonate, and then autoclaving the moist mixture for 6 hours at 15 pounds pressure. The autoclaved yeast was later dried and ground to a fine powder. A daily dose of 0.3 gm. of this product was found to supply adequate vitamin G when fed as the only source of this vitamin.

The control diet was similar in composition to that which had previously been used in this laboratory in vitamin B and vitamin G studies. It consisted (in parts per 100) of washed casein 18, salt mixture (McCollum 185) ('18) 4, agar 2, sucrose 20, cod liver oil 2, butter fat 3, and dextrin 51. A diet of this composition was chosen, because it offered possibilities of quite wide variations in its constituents at the expense of the sucrose. This quality was highly desirable, since sucrose, a fairly pure and uniform source of carbohydrate, was selected as the variable compensator in the variations of the other constituents of the diet. This diet alone, and in combination with daily allotments of the vitamin B concentrate, the vitamin G fraction, and a combination of these two preparations, was fed to groups of depleted rats at intervals during the progress of the investigation. These tests were carried out in order to test the adequacy of the daily dosage of the two vitamins, and to show that definite growth responses could be produced by feeding rats a diet of this composition, when it is properly supplemented.

Using a diet of this composition as a basis, five series of diets, in each series of which one of the above ingredients was varied systematically within practical limits, were fed to respective groups of rats during a three-phase experimental period throughout which the animals received 1) neither vita-

min B nor vitamin G, 2) vitamin B, and 3) vitamins B and G. In addition, a sixth series (of four diets), in which 2, 4, 6, and 8 per cent of CellU flour were used as sources of roughages instead of agar, was similarly tested. In order to conserve space, the number and composition of the various diets used are presented in table 1.

The components of the various diets were of the quality that is most commonly used in nutritional work of this type. The casein was a good grade South American product. It was extracted with both acidulated water and 95 per cent alcohol until practically free of the vitamin B complex. All ingredients used in the salt mixtures were of the C.P. grade except the ferric citrate and the calcium lactate. These salts were of the U.S.P. grade. The ager was a U.S.P. powder obtained from a reliable chemical supply company. Refined sucrose of the usual commercial grade was used. The Crisco was purchased from a local grocery. The cod liver oil was the E. L. Patch medicinal product. The butter fat was prepared in the usual manner from fresh creamery butter purchased from the college creamery. The dextrin was prepared from commercial cornstarch by moistening the starch with a dilute solution of citric acid and autoclaving for 4 hours at 15 pounds pressure. The CellU flour was a highly purified form of cellulose furnished through the courtesy of the Chicago Dietetic Supply House, Inc. All diets were made up at weekly intervals and were stored in an electric refrigerator.

DATA

While close observation was maintained upon all animals during the course of the investigation and a fairly complete record was kept at all times, it is believed that the various differences in growth responses manifested by the several groups of rats receiving the different diets are the most accurate indices of the rat's requirement for vitamin B and for vitamin G while subsisting on these various dietaries. For this reason and also to conserve space, the data obtained in the several series of experiments have been condensed and presented in table 2 and in figures 1 to 8, inclusive.

TABLE 1

Showing the number and the composition of the various diets used

DIET NO.	CRISCO	SALTS	CASEIN	AGAR AGAR	CELLU FLOUR	DEXTRIN	SUCROSE	COD LIVER OIL	BUTTER FAT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
306[1]	0	4	18	2	0	51	20	2	3
324	0	4	18	2	0	51	25	0[2]	0[2]
319[1]	0	4	18	2	0	51	20	2	3
320	5	4	18	2	0	51	15	2	3
321	10	4	18	2	0	51	10	2	3
322	15	4	18	2	0	51	5	2	3
323	20	4	18	2	0	51	0	2	3
328	0	1	18	2	0	51	23	2	3
325	0	2	18	2	0	51	22	2	3
329[1]	0	4	18	2	0	51	20	2	3
326	0	6	18	2	0	51	18	2	3
327	0	8	18	2	0	51	16	2	3
330	0	10	18	2	0	51	14	2	3
331	0	4	8	2	0	51	30	2	3
332	0	4	12	2	0	51	26	2	3
333	0	4	16	2	0	51	22	2	3
334	0	4	20	2	0	51	18	2	3
335	0	4	24	2	0	51	14	2	3
336	0	4	28	2	0	51	10	2	3
337	0	4	18	0	0	51	22	2	3
338[1]	0	4	18	2	0	51	20	2	3
339	0	4	18	4	0	51	18	2	3
340	0	4	18	6	0	51	16	2	3
341	0	4	18	8	0	51	14	2	3
342	0	4	18	10	0	51	12	2	3
345	0	4	18	0	2	51	20	2	3
346	0	4	18	0	4	51	18	2	3
347	0	4	18	0	6	51	16	2	3
348	0	4	18	0	8	51	14	2	3
353	0	4	18	2	0	0	71	2	3
352	0	4	18	2	0	11	60	2	3
351	0	4	18	2	0	31	40	2	3
350[1]	0	4	18	2	0	51	20	2	3
349	0	4	18	2	0	71	0	2	3

[1] These diets all had the same composition but were designated under different numbers, since they were used as control diets in different series and at different times.

[2] Crystalline carotene and 10,000 D viosterol were dissolved in petroleum ether and added to the basal diet at weekly intervals in sufficient quantities to furnish 10 Sherman units of vitamin A and 10 A.D.M.A. units of vitamin D for each gram of food.

Showing the number of animals considered, their average initial weight, their gain in weight, their food intakes, and the approximate calorie equivalent per gram gain in weight

Showing the number of animals considered, their average initial weight, their gain in weight, their food intakes, and the approximate calorie equivalent per gram gain in weight

DIET NO.	VARIABLE CONSTITUENT	PER CENT VARIABLE CONSTITUENT PRESENT	NUMBER OF ANIMALS CONSIDERED	AVERAGE INITIAL WEIGHT	GAIN DURING DEPLETION PERIOD	GAIN DURING B PERIOD	GAIN DURING B + G PERIOD	TOTAL GAIN IN WEIGHT	WEEKLY FOOD INTAKE DURING THE DEPLETION PERIOD	WEEKLY FOOD INTAKE DURING THE B PERIOD	WEEKLY FOOD INTAKE DURING THE B + G PERIOD	APPROXIMATE CALORIC INTAKE PER GRAM INCREASE IN WEIGHT [1]		
												During depletion period	During the B period	During the B + G period
				gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.			
306	Unsupplemented		9	42	7	..	..	7	23	..	..	42	..	..
306	Vitamin B concentrate		18	41	7	17	..	24	23	21	..	42	39	..
306	Autoclaved yeast		10	41	8	..	9	17	23	..	17	33	..	..
306	Vitamins B and G		9	42	6	..	96	102	23	..	32	46	..	11
324	Fat	0	5	40	−2	14	34	46	16	18	24	..	37	22
319	Fat	5	5	41	3	13	35	51	17	19	26	69	47	26
320	Fat	10	5	42	4	8	44	56	19	16	25	60	50	21
321	Fat	15	5	40	4	12	50	66	19	17	27	64	51	21
322	Fat	20	5	41	4	14	59	77	18	18	31	64	49	21
323	Fat	25	5	40	6	11	48	65	17	16	25	53	58	23
328	Inorganic	1	5	43	−1	20	46	65	20	24	31	..	30	21
325	Inorganic	2	6	42	10	17	34	61	22	23	28	27	33	25
329	Inorganic	4	6	41	9	11	26	46	22	22	24	29	48	28
326	Inorganic	6	6	41	11	8	25	44	21	17	23	23	50	28
327	Inorganic	8	5	42	10	5	23	38	21	16	23	24	74	30
330	Inorganic	10	6	43	6	0	15	21	21	16	22	40	..	44
331	Casein	8	7	41	−3	8	37	42	21	24	27	..	96	19
332	Casein	12	10	41	4	11	42	57	22	27	29	66	79	18
333	Casein	16	9	40	7	13	36	56	22	26	29	38	64	21
334	Casein	20	10	40	8	12	40	60	23	25	28	35	67	18
335	Casein	24	10	41	7	14	38	59	22	24	29	38	55	20
336	Casein	28	6	40	4	16	29	49	20	23	28	60	46	25
337	Agar	0	10	40	4	17	34	55	24	23	29	72	33	21
338	Agar	2	10	41	7	16	36	59	25	25	31	43	38	22
339	Agar	4	9	41	10	22	33	65	26	27	34	31	30	26
340	Agar	6	10	42	13	19	38	70	29	29	35	27	37	24
341	Agar	8	10	41	17	22	33	72	26	28	38	18	31	29
342	Agar	10	10	42	16	23	37	76	25	28	37	19	28	25
345	CellU flour	2	6	40	7	20	32	54	22	26	31	38	31	24
346	CellU flour	4	6	41	13	20	33	66	22	27	34	20	33	27
347	CellU flour	6	6	41	13	22	33	68	23	26	38	21	28	29
348	CellU flour	8	6	42	19	25	30	74	25	32	36	16	31	30
353	Dextrin	0	4	42	−7	2	21	16	19	15	19	..	181	24
352	Dextrin	11	5	43	−6	2	19	15	19	16	18	..	192	25
351	Dextrin	31	6	41	−6	3	36	33	20	17	23	..	136	17
350	Dextrin	51	6	42	−3	8	41	46	21	19	27	..	57	19
349	Dextrin	71	6	42	5	14	46	65	23	22	31	55	38	17

[1] Calculated on the basis that protein = 4 cal. per gram, carbohydrate = 4 cal. per gram, fat = 9 cal. per gram, and that casein and yeast are 100 per cent protein.

DISCUSSION

In compiling the above data, records of individual animals which departed abnormally from the group averages were omitted, and only those animals were considered which reacted more or less uniformly as a group. The number of animals which failed to meet this requirement was not large, but did appear to be greater in certain experimental groups. In most cases, these abnormal responses seemed to have followed an excessive period of depletion during the first stage of the experiment. Most of these animals had manifested marked symptoms of beriberi before the end of the 21-day depletion period, and did not make the immediate characteristic response when the daily allotment of the vitamin B concentrate was added. It appears most probable that more uniform growth responses would have been obtained in certain groups of rats had this depletion period been reduced by several days. There is, on the other hand, some evidence which indicates that groups of rats receiving certain diets, could have withstood a more prolonged depletion period without serious consequence.

During the course of the investigation, a total of forty-six animals were used in testing the basal diet and the effectiveness of the vitamin B and the vitamin G fractions in supplementing this diet. The data obtained (fig. 1) show that, a) all rats that received the basal diet unsupplemented died between the twenty-ninth and the fifty-ninth days, b) seventeen of the eighteen rats that received the basal diet supplemented by the vitamin B concentrate lived through the 8-week experimental period, c) nine of the ten animals that received the basal diet supplemented with the vitamin G fraction died before the end of the experiment, and d) those animals that received the basal diet supplemented by both the vitamin B concentrate and the vitamin G fraction made an average gain of 11 gm. per week during the 8-week period.

It was interesting to note in connection with the control diet that, while the composition of the diet remained unchanged from beginning to end, the first animals to die mani-

fested no apparent symptoms of beriberi other than an unsteady gait, and when found dead they were usually clutched to the cage by paws and teeth. Those animals which survived longer, however, manifested very characteristic symptoms several days previous to death. These facts, together with observations made in connection with other experiments, suggest that some relation must exist between the age of the rat and the type of vitamin B deficiency symptoms manifested.

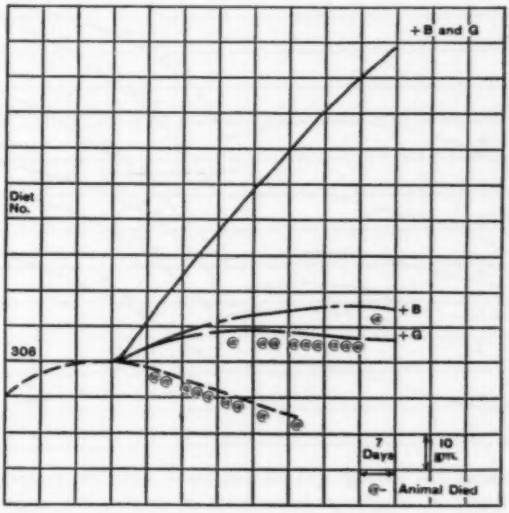

Fig. 1 Showing the effect of supplementing the basal diet (306) with 0.1 ml. of vitamin B concentrate and 0.3 gm. of the vitamin G fraction, separately and in combination.

In a consideration of the data obtained from the groups of rats receiving the diets comprising the fat series (fig. 2), several interesting trends become apparent and seemed to justify some consideration. Diet 324 had been made practically fat free by extracting the properly combined ingredients with ethyl ether for 16 hours in a continuous extractor. Sufficient crystalline carotene and 10,000 D viosterol were added to the ether-free diet to furnish 10 units each of vitamin A

and vitamin D for each gram of food. By using highly potent sources of these vitamins, the fat content of the diet was maintained at the lowest practical level. By increasing the fat content of this diet first by replacing a portion of the sucrose by cod lived oil and butter fat in order to supply the fat soluble vitamins, and then by further replacement of the

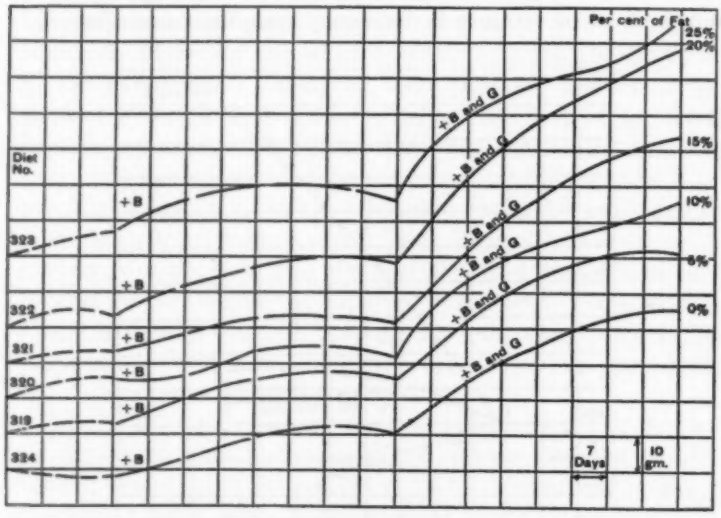

Fig. 2 Showing the average growth responses made by groups of rats receiving 1) vitamin B complex deficient diets of varying fat content; 2) these diets supplemented with daily allotments of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented daily with both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

sucrose with 5, 10, 15 and 20 per cent of Crisco, a series of diets were made up that ranged in fat content from 0 to 25 per cent.

The data obtained through feeding this series of diets to groups of young rats emphasizes three points worthy of note. a) Rats receiving diets containing appreciable quantities of fat (Crisco) were more difficult to deplete of their vitamin B stores than similar animals receiving diets of low fat con-

tent. b) The data do not indicate that the fat content of the diet bears any relation to the rat's requirement for vitamin G. c) When restricted daily allotments of both vitamins B and G were fed to rats, greater growth rates were obtained from those animals receiving diets containing from 15 to 20 per cent of fat than from similar animals receiving corresponding diets of lower fat content.

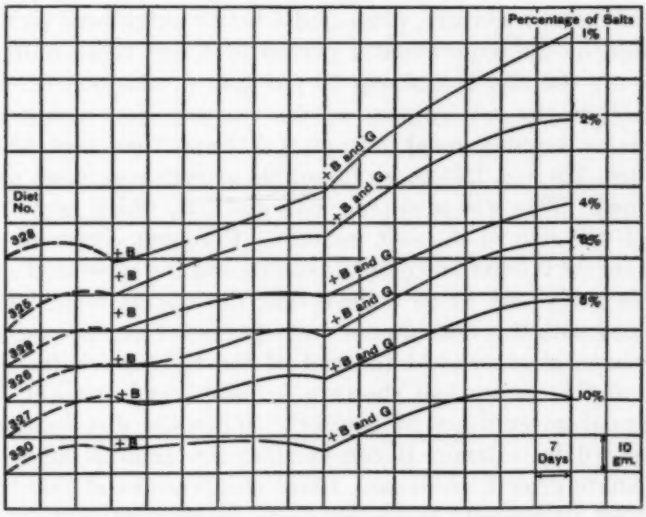

Fig. 3 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying salts content; 2) these diets supplemented with daily dosages of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented with daily dosages of both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

The results obtained with the series of diets in which the salt mixture was the variable constituent under consideration likewise revealed some points of interest (fig. 3). The group of rats which received diet 330 containing 10 per cent of the salt mixture made less than the usual growth response during the 3-week depletion period. In this case the vitamin B concentrate appeared to have little or no supplementing

value. When this diet was further supplemented by autoclaved yeast, the animals grew at a very low rate during the next 5 weeks. During the last 2 weeks that this group of animals was under observation, increased evidence of nutritional failure became apparent. It was highly improbable if any would have lived for 2 additional weeks had the experimental period been extended.

The group of rats receiving diet 327, which contains 8 per cent of the salt mixture, presented a better nutritional picture throughout the experimental period than did those animals receiving the diet containing 10 per cent of this constituent. In fact, as the salt content of the various diets composing the series was decreased through 8, 6, 4 and 2 per cent (diets 327, 326, 329 and 325) more favorable growth responses were obtained. This was also true with diet 328, which contained only 1 per cent of the salt mixture. Frequent observations, however, of the various animals composing the several groups of this series led us to believe that the results which were obtained with this diet are not entirely free of an unsuspected experimental error. At the end of the third week the animals of this group had shown a definite loss in weight, but presented no evidence of beriberi. When the diet was supplemented by vitamin B concentrate, the animals made an immediate growth response. Close observations at this time revealed all animals of the group to be consuming their excreta, especially the urine. This tendency was evidently acquired about the end of the depletion period and was continued throughout the experiment. Consequently, the data obtained on diet 328 are difficult to evaluate in terms of either vitamins B and G or salts intake. The practice of coprophagy was seldom observed among the other groups of animals comprising this series. It is believed, therefore, that the practice in this particular group was an attempt on the part of the experimental animals to maintain a favorable mineral balance while receiving a diet inadequate in inorganic constituents.

When a series of diets, in which the protein constituent (casein) was varied in 4 per cent intervals from 8 to 28

per cent, were fed to groups of rats receiving controlled daily allotments of vitamins B and G, under comparable conditions, no startling differences in growth responses were obtained (fig. 4). The group of rats which received diet 331 containing 8 per cent of casein lost weight during the first 3 weeks that they were on experiment. While none of the animals

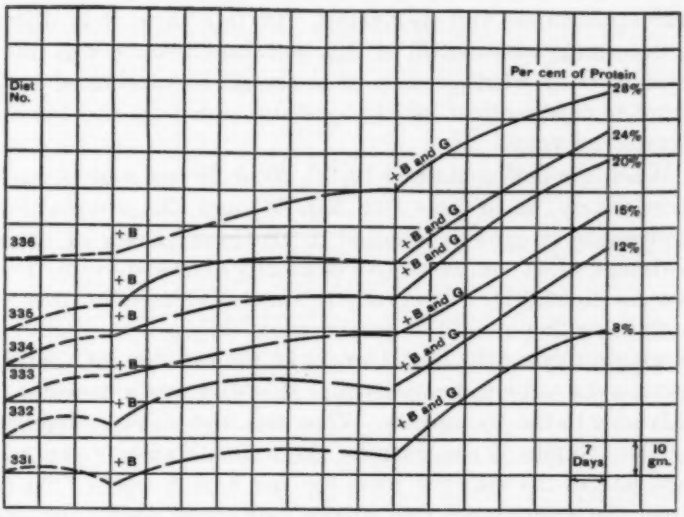

Fig. 4 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying protein content; 2) these diets supplemented daily with 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented by both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

of this group died from beriberi during this time, some of them did exhibit marked paralytic symptoms. Later, three of the ten animals comprising the group were found to be practicing coprophagy; consequently they were removed from the experiment. When a daily allotment of the vitamin B concentrate was given to each animal of this group, a slight gain in weight amounting to 10 gm. resulted during the next 5 weeks, part of which was subsequently lost. The animals

of this group, after being depleted for 3 weeks, and then receiving vitamin B for 8 weeks, did not show any severe symptoms of vitamin G deficiency. There was the usual roughness of hair, scaly feet, and some local depilation of head and body. When the diet was further supplemented with autoclaved yeast, each animal of the group made a very definite growth response and improved markedly in both general appearance and well-being. In this case, it is difficult to determine whether all of this increased growth was due to vitamin G or whether part of it should be attributed to the supplementing effect of the protein added in the form of autoclaved yeast.

When the diet contained 12, 16, 20 or 24 per cent of casein, respectively, as in diets 332, 333, 334 and 335, a definite improvement in growth resulted in all three phases of the experiment when compared to the results obtained on the lower protein diet (331). The group of rats which received diet 336, containing 28 per cent of casein, made only a very slight gain in weight during the first 3 weeks of the experiment. At first there were some indications that this diet was somewhat unpalatable to the young rats. When this diet was supplemented by the vitamin B concentrate, the animals grew at an average rate of 2.5 gm. per week for the next 8 weeks. At this time, the animals of this group had made about the same increase in weight as the groups receiving diets 333, 334 and 335. On further supplementing diet 336 with autoclaved yeast, these animals increased their average rate of growth to 5 gm. per week for the next 6 weeks, which was less than the growth rates of several other groups during this same period.

The data obtained through feeding rats this series of diets indicate that, within the limits of the usual experimental diet, slight difference in protein has no measurable effect upon the rat's requirement for vitamins B and G. When the protein content of the diets is increased or decreased beyond certain limits, other complications arise which make it more difficult to interpret the experimental results obtained.

When a series of six diets, in which agar was the variable constituent, were fed to respective groups of rats, some noticeable differences were observed in the resultant growth responses (fig. 5). The ten animals that received diet 337, which contained no agar, made a slight gain in weight during the first 2 weeks of the depletion period, but part of this gain was later lost during the third and last week of the period.

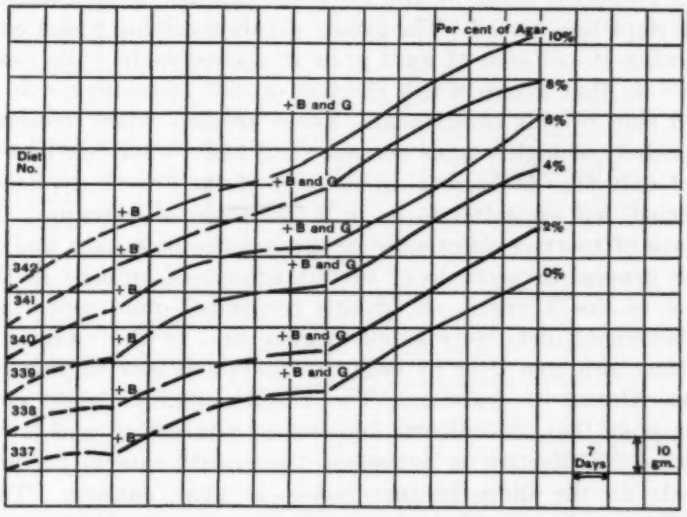

Fig. 5 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying agar content, 2) these diets supplemented with 0.1 ml. daily allotments of the vitamin B concentrate, and, 3) these diets supplemented with both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

At this time all animals of the group appeared to be in an unthrifty condition and some of them showed definite paralytic symptoms. On supplementing this diet with the vitamin B concentrate, all animals reassumed growth for the next 5 weeks. Ruffled fur and retarded growths appeared to be the only visible external symptoms of vitamin G deficiency. On further supplementing the diet with autoclaved yeast, all animals of the group made a uniform and uneventful growth of 5 gm. per week for the next 6 weeks.

When the diet contained 2 per cent of agar (diet 338), the growth response, in general, was very similar to that obtained on the agar-free diet. The only noticeable difference was a smaller loss in weight during the depletion period and fewer symptoms of beriberi during this time. As the agar content of the diets was further increased in 2 per cent intervals, as in diets 339, 340, 341 and 342, marked differences appeared in the responses made by the respective groups of rats during the depletion period. The group of rats receiving a diet containing 10 per cent of agar grew at approximately the same rate as that group which received a diet containing only 8 per cent of this constituent. Those animals which received the diets of higher agar content (6, 8 and 10 per cent) were not only free of beriberi at the end of the 3-week depletion period, but appeared to be in a fair state of nutrition. In spite of marked differences in the growth responses and in the general appearance of the six groups of animals at the end of the 3 weeks, all groups responded quite uniformly when these diets were supplemented, first by the vitamin B concentrate and later by both the vitamin B concentrate and the vitamin G fraction. The results indicate quite conclusively that the uniform addition of vitamins B and G became less effective in increasing the growth rate of the animals as the diets became richer in agar content. This difference in supplementing value was more marked in the case of the vitamin B concentrate.

The above data in themselves were not sufficient to justify the postulation of a specific mechanism by which the vitamin B and the vitamin G requirement of the rat could be affected by the quantity of agar incorporated in the diet. A number of different possibilities suggested themselves, but none appeared tenable without further data. To us the above data appeared to be open to criticism on the ground that an impure or an unrefined agar had been used in the various diets. We anticipated considerable difficulty in attempting to free the agar from impurities by any mode of extraction with vitamin solvents. A more logical plan appeared to be a repetition

of at least part of the experiments involving the agar-containing diets, using CellU flour instead of agar as a source of roughage.

In the CellU flour series, only four diets were fed. Diets 345, 346, 347 and 348 (fig. 6) contained 2, 4, 6 and 8 per cent, respectively, of CellU flour and in other respects were identical in composition to diets 338, 339, 340 and 341 of the agar

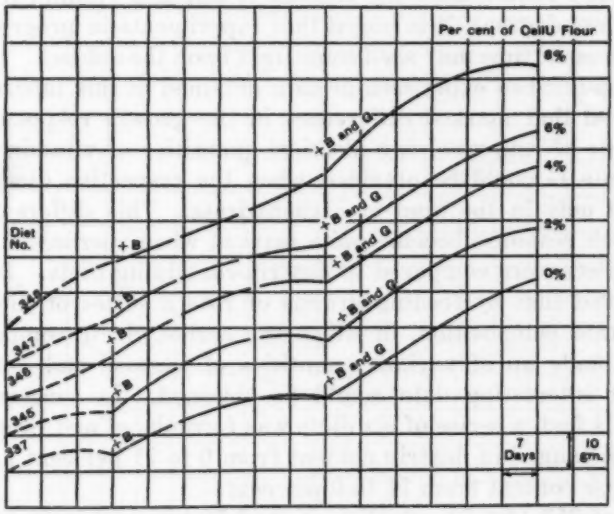

Fig. 6 Showing the average growth responses made by groups of rats receiving, 1) vitamin B complex deficient diets of varying CellU flour content, 2) these diets supplemented by daily additions of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented by daily additions of both 0.1 ml. of vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

series. The test with diet 337 was not repeated at this time. The growth curve presented is the same as presented in figure 5. Four groups of rats of six animals each were fed the CellU flour diets under conditions as nearly identical as possible to that maintained in the agar series. A comparison of figures 5 and 6 will reveal the striking similarity in the two series of results obtained. The nutritional pictures presented by the two groups of rats, one group of which re-

ceived a diet containing a definite percentage of agar, while the other group received a diet containinng an equal percentage of CellU flour, were so similar in all respects that a rediscussion appears unnecessary at this time.

While no satisfactory explanation can be offered at this time relative to the physiological mechanism by which the fiber (agar and CellU flour) content of the diet influences the rat's requirement for vitamin B and also vitamin G (but to a less degree), it is hoped that experiments in progress at the present time may shed some light upon the subject.

Unpublished experimental data obtained in this laboratory showed that marked differences in the growth responses of groups of rats receiving identical quantities of vitamin B or vitamin G could be obtained when the respective diets differed only in the kind of carbohydrate. This difference in growth response became quite marked when sucrose-containing diets were compared to dextrin-containing diets. It was believed that by feeding groups of rats a series of diets of constant composition, in which the carbohydrate component was made up of various quantities of sucrose and dextrin, other interesting data would be obtained. In consequence of this fact, a series of six diets was formulated and prepared which ranged in dextrin content from 0 to 71 per cent and in sucrose content from 71 to 0 per cent.

Diet 353 (fig. 7), which contained 71 per cent sucrose and no dextrin, was fed to a group of eight animals. Each animal of the group lost in body weight from the beginning, and by the seventeenth day two of the eight animals were dead. The feeding of the vitamin B concentrate was started at this time, as it was thought that the remaining animals could not survive a 21-day depletion period on the diet and yet retain sufficient vitality for further use in the feeding test. Two more animals died, one on the eighteenth and the other on the twenty-first day. The remaining animals made a definite growth response as the result of receiving the daily allotment of the vitamin B concentrate. The growth-stimulating effect lasted for about 3 weeks when the four surviving animals

began to lose weight gradually for a period of 3 weeks or until the diet was further supplemented by daily additions of autoclaved yeast. During the 2 weeks previous to the addition of the autoclaved yeast, all four of the animals manifested some of the external symptoms usually associated with vitamin G deficiency in the rat. These symptoms include salivation, ulceration of the lining of the mouth and tongue,

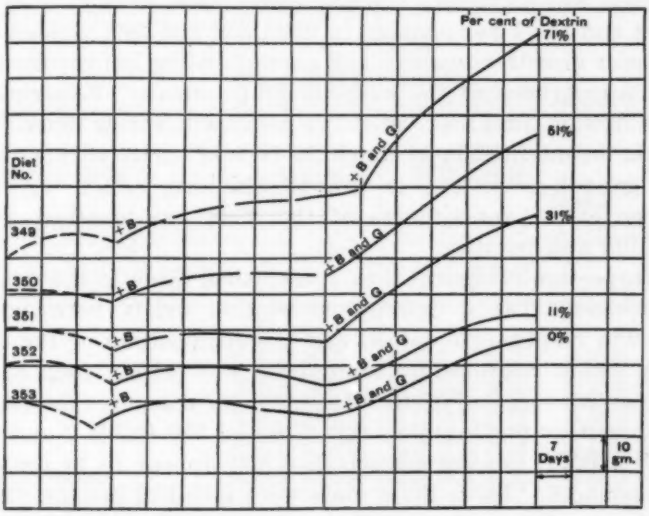

Fig. 7 Showing the average growth responses made by groups of rats receiving, 1) vitamin B deficient diets of varying dextrin content, 2) these diets supplemented by daily allotments of 0.1 ml. of the vitamin B concentrate, and, 3) these diets supplemented with daily allotments of both 0.1 ml. of the vitamin B concentrate and 0.3 gm. of the vitamin G fraction.

inflamed feet and tail, frequently followed by some signs of necrosis, alopecia areata, and distinct diarrhea. When 0.3 gm. of autoclaved yeast was given to each animal daily, there was some delay in the resumption of growth, but the external symptoms showed marked improvement by the end of the first week. From that point, the animals grew at a fairly uniform rate for the remaining 5 weeks and improved in both activity and general appearance.

On decreasing the sucrose content of the diet to 60 per cent and adding 11 per cent of dextrin as in diet 352, the nutritional picture in general was not greatly improved. The most noticeable difference occurred during the preliminary depletion period. When the sucrose content of the diet was further reduced to 40 per cent and the dextrin increased to 31 per cent, as in diet 351, a distinctly improved nutritional picture was obtained. Further increases of dextrin to 51 per cent and to 71 per cent, as in diets 350 and 349, yielded still greater growth responses and corresponding improvement in the appearance of the experimental animals. Experiments are now in progress which we hope will throw some light upon the mechanism by which the type of carbohydrate exerts its growth-promoting or growth-inhibiting effect, while all other known constituents of the diets are identical as to quality and quantity.

Inspection of figures 2 to 7, inclusive, leads to the general conclusion that a definite correlation exists between the growth responses obtained and the composition of the diets fed. This relationship holds quite well within each of the respective series of diets. But when one compares the results obtained by feeding diets 329, 338 and 350 (table 2 and figs. 3, 5 and 7), this correlation does not appear to be quite so pronounced. These three diets were identical in composition and were fed to three different groups of rats under very similar conditions, except the season of the year in which the experiments were carried out. Diets 306 and 319 were also identical in composition to the above diets but each was fed under different conditions and for this reason the results are not comparable to the results obtained on the above diets.

Consideration of the approximate caloric intake of the various groups of rats during the three phases of the experiments shows that the vitamin B concentrate alone was not always effective in reducing the energy required to produce a unit of growth. In fact, no definite relationship appeared to exist between the quantity of ingested energy and the resultant

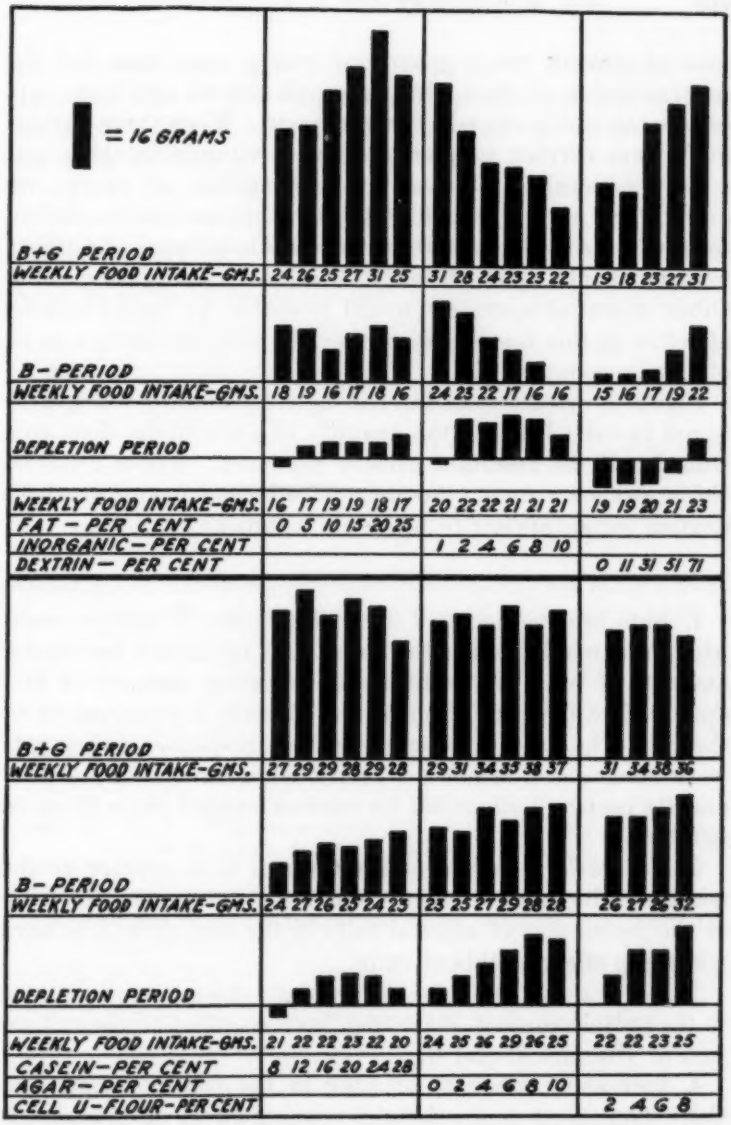
= 16 GRAMS
B+G PERIOD
WEEKLY FOOD INTAKE-GMS. 24 26 25 27 31 25 31 28 24 23 23 22 19 18 23 27 31
B- PERIOD
WEEKLY FOOD INTAKE-GMS. 18 19 16 17 18 16 24 23 22 17 16 16 15 16 17 19 22
DEPLETION PERIOD
WEEKLY FOOD INTAKE-GMS. 16 17 19 19 18 17 20 22 22 21 21 21 19 19 20 21 23
FAT - PER CENT 0 5 10 15 20 25
INORGANIC - PER CENT 1 2 4 6 8 10
DEXTRIN - PER CENT 0 11 31 51 71
B+G PERIOD
WEEKLY FOOD INTAKE-GMS. 27 29 29 28 29 28 29 31 34 35 38 37 31 34 38 36
B- PERIOD
WEEKLY FOOD INTAKE-GMS. 24 27 26 25 24 25 23 25 27 29 28 28 26 27 26 32
DEPLETION PERIOD
WEEKLY FOOD INTAKE-GMS. 21 22 22 23 22 20 24 25 26 29 26 25 22 22 23 25
CASEIN - PER CENT 8 12 16 20 24 28
AGAR - PER CENT 0 2 4 6 8 10
CELL U - FLOUR - PER CENT 2 4 6 8

Fig. 8 Showing the relation of the quantity of the various diets consumed to the resultant growth responses made during the three phases of the feeding trials.

rate of growth when groups of young rats were fed the several series of diets, first unsupplemented and then supplemented with a vitamin B concentrate. When these various diets were further supplemented with vitamin G, there was a very noticeable decrease in the quantities of energy required for a unit of growth. The data obtained are insufficient to justify the assumption that vitamin G is unique in its ability to bring about a more efficient utilization of energy for growth. Other essential nutrients would probably be found equally effective in this respect, were they the limiting factors in an otherwise complete diet.

Figure 8 reveals some interesting relationships which were found to exist between the quantity of the various diets consumed and the resultant growth produced. These relationships, as shown among the diets of both the inorganic and the dextrin series, appear to be highly significant.

CONCLUSIONS

1. Rats became depleted of their vitamin B reserve somewhat less readily when the basal diet contained increasing amounts of fat. The addition of increasing amounts of fats appeared to have no effect on the vitamin G requirement of the rat. The most satisfactory growth responses (when rats were receiving a somewhat restricted intake of vitamins B and G) occurred when the fat content ranged from 15 to 20 per cent.

2. The rat's requirement for vitamin G is greater as the mineral salts are increased. And, conversely, the lowering of the percentage of mineral salts in the diet seemed to have a sparing effect on this vitamin.

3. No evidence could be obtained to show that variations in the protein content of the diet have an effect on the utilization of vitamins B and G.

4. Increasing amounts of fiber in the form of agar and CellU flour possessed a definite sparing effect on vitamins B and G utilization. The beneficial effect of fiber is thought to be due to the production of more favorable conditions for the growth of microorganisms in the digestive tract.

5. The demand of the rat for vitamins B and G increases when sucrose is fed as the sole source of carbohydrate, while the need for these vitamins decreases as the sucrose is replaced by dextrin.

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THE EFFECT OF A HIGH FAT MEAL ON THE RESPIRATORY QUOTIENT AND HEAT PRODUCTION OF NORMAL AND OBESE INDIVIDUALS[1]

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TWO FIGURES

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Many will agree that not all of the problems concerned with the subject of body weight are settled, although the ideas of some students of obesity are put forth in a manner indicating finality. When accounting for the production of obesity, one finds a difficulty in integrating the various theories as to etiology, abnormal physiology and the role of ductless glands and constitution. The many clinical classifications only add to the confusion.

The concept that body weight depends entirely upon the balance between food eaten and the outflow of energy carries with it the implication that all individuals are potentially thin or fat in accordance with that relationship. Commonplace observations make the unqualified acceptance of such a law difficult. If obesity is wholly exogenous, it would be necessary to consume only the equivalent of one patty of butter daily in addition to the needs of the body in order for an individual to become obese in a few years, as has been pointed out by Dubois ('27). Von Noorden stated that the excess consumption of 200 calories daily would lead to the deposition of 7.8 kg. of fat in a year's time.

[1] This work was made possible by the generous gift of the equipment for a metabolism laboratory by the late Mrs. William H. Crosby.

The painstaking quantitative experiments of Newburgh and his co-workers ('30) have shown that the laws of conservation of energy are immutable when applied to the energy balance of obese individuals. Their work ('31) also reflects great doubt upon the 'luxus konsumption' theory of Grafe. They have, also shown, as has been suspected, that the failure of some obese individuals to lose weight while taking a sub-caloric diet, is caused by a temporary hydration of the body. In this sense obesity must be considered exogenous, but the possibility that fat people possess an inherent qualitative metabolic reaction, which is not present in normal individuals, is, we believe, entirely reconcilable with such a concept. Observations on large groups of obese people by Von Noorden, Bauer, and Lyon et al. ('32) show fatness to be a family characteristic in a very large majority of instances. That is, some people may have either as a constitutional or an acquired characteristic, the tendency to convert foodstuffs into fat and store it more readily than others. If the term 'endogenous' be reserved to imply such a reaction, then it would be misleading. Both of these terms, however, are unfortunate as they attempt to differentiate states that are not easily separated clinically, or by laboratory methods.

The storage of small amounts of fat either preformed or converted from carbohydrate has been difficult to measure by short term investigations. Long term observations, which necessitate the measurement of the energy consumed during activity, are quite beyond the patience of either the investigator or the subject. Thus, the question of the nature of obesity has been the target for many opinions and theories which too often have been supported only by a few suggestive observations.

Whether obesity belongs to the so-called constitutional or endocrine diseases, or both, does not essentially alter the problem of its pathologic physiology, unless the clinical types can be clearly separated by some kind of measuring stick. Current opinions concerning this are so controversial and diverse that any discussion of this phase seems impractical.

(Silver and Bauer, '31; Jarløv, '32). In the present study no attempt has been made to make any such differentiation.

The primary purpose of this study was to observe any difference in the metabolic reactions of normal and obese individuals to food. In a preliminary study a few subjects were observed as to the effect of glucose, protein and fat separately. Since one of the first obese subjects studied showed a consistent and a considerable rise in the respiratory quotient after a fat meal, and since the responses to protein and glucose were not dissimilar in the first few normal and obese subjects, it seemed advisable to concentrate our observations on the effect of a fat meal.

The subjects in this study were chosen from both clinic and private practice. The majority were cases of so-called simple obesity; one (J. S.) might be classified as a case of cerebral obesity as her gain followed encephalitis. All observations were made while they were living as usual and taking their accustomed meals. The majority of tests were made at a time when the subjects were out-patients. The weights of the obese subjects varied from 56 per cent to 169 per cent above the average normal—average 81 per cent. In the obese diabetic group the variation was from 14 per cent to 143 per cent above normal—average 47 per cent.

A rest period of at least an hour was allowed. Nearly all of the subjects had previously had one or more metabolism tests and a few had been observed through extended metabolic studies.

The fat meal consisted of 50 gm. of butter on a few bran wafers, which served as carriers, 50 gm. of butter in about 250 cc. of clear chicken broth and 50 gm. of mayonnaise on 50 gm. of lettuce. Fat, 128 gm.; protein, 2.7 gm.; carbohydrate, trace.

This meal was given immediately after satisfactory post-absorptive (14 to 16 hours after food) quotients had been obtained. Absorptive quotients were then determined about each hour for a period of 6 to 8 hours following the ingestion of the fat meal. The subjects were occasionally allowed to

walk to the lavatory, sit up in bed, read and talk after the completion of a period, but were required to rest quietly for at least 20 minutes before the next period. The length of the periods was from 10 to 15 minutes, except in the early stage, when the metabolism of a few subjects was determined for only 6-minute intervals.

The expired air was collected in the Tissot-Boothby Gasometer and analyzed by the Haldane apparatus in duplicate. Subjects whose ventilation volumes were unsteady or whose carbon dioxide percentages in the expired air were erratic, were eliminated. This feature was closely guarded, so that quotients would be unaffected by changes in ventilation.

The Boothby and Sandiford modification of the DuBois standards was used in the calculation of the basal metabolic rates.

The fasting respiratory quotient. This varied in the twelve normal subjects (six average and six below average weight) between 0.76 and 0.88 (table 1), the average being 0.825, which corresponds to the usual average post-absorptive quotient stated by DuBois ('27). Joslin ('23) calculated the quotients of 236 of 239 normal individuals and determined the average to be 0.83. Benedict ('19) found the average quotient of sixty-one readings to be 0.795. In the work of Lyon, Dunlop and Stewart ('32) on seven normal, healthy women students, who were accustomed to metabolism tests, an average quotient of 0.797 was found.

In the obese group, twenty subjects, the variation was between 0.72 and 0.83, the average being 0.765. Five were 0.80 or above. Of the eleven obese diabetic patients the quotient varied from 0.73 to 0.785, with only one above 0.80—0.83. The average was 0.767. The diabetes was under control by diet in all of the latter group at the time the observations were made. One was taking insulin, but did not have it on the day of the test. The diets had been about 100 gm. of carbohydrate, a gram of protein per kilogram of body weight, and were designed so the patients would either maintain their weight or lose weight slowly.

TABLE 1

Normal subjects

Subject	Age	Weight Kg.	Height Cm.	Sex	B.M.R. per cent.	Basal Period			First Period Av. 70 min.			Second Period Av. 140 min.			Third Period Av. 220 min.			Fourth Period Av. 305 min.			Fifth Period Av. 415 min.		
						Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.
H.K.	16			F		166	0.85		186	0.85		189	0.81		181	0.82		178	0.85				
W.H.	20	68	175	M	97	249	0.86	40.0	283	0.80	44.9	275	0.80	43.6	265	0.79	41.6	277	0.76	43.4			
W.F.	19	74	177	M	95	265	0.82	39.9	292	0.78	44.2	293	0.80	44.5	299	0.79	45.2						
L.S.	27	77	165	M	96	241	0.86	38.6	271	0.86	43.5	279	0.85	44.5	281	0.75	43.7	260	0.81	41.1	247	0.82	39.2
C.T.	45	77	180	M	102	265	0.79	38.5	300	0.79	44.0	301	0.80	44.2				289	0.75	41.8			
D.G.	25	57	156	F	98	192	0.87	36.5	237	0.75	43.4				229	0.79	42.5	217	0.84	40.7	229	0.77	42.2
F.B.	22	45	150	F	89	156	0.82	35.1	188	0.85	36.0	180	0.84	34.2	169	0.84	36.1						
S.W.	37	46	165	F	92	175	0.85	35.1	197	0.77	37.7	177	0.86	35.1	186	0.86	36.7	188	0.80	36.6	192	0.87	37.9
E.L.	26	50	166	F	96	190	0.76	35.4	196	0.81	37.3	208	0.81	39.5	209	0.79	39.2	218	0.75	40.2			
D.R.	24	48	156	M	90	194	0.84	36.8	202	0.86	40.5	215	0.82	42.4	195	0.79	38.5	222	0.79	43.8			
E.C.	25	51	169	F	107	215	0.80	39.6	225	0.84	41.4	215	0.79	39.4	216	0.75	38.9	240	0.77	45.7			
E.B.	56	45	170	F	91	159	0.84	31.0	161	0.86	31.5	175	0.77	35.1	179	0.85	34.9	188	0.78	36.2	195	0.78	37.1

The effect of the fat meal on the respiratory quotient. Of the group containing six of average weight and six who were below (i.e., 'normal') there was no essential difference in the response to a fat meal. There was no change in the average respiratory quotient at the end of an hour. At the second hour the respiratory quotient was depressed (average 0.80), where it remained to the fifth hour. At the sixth hour there was a beginning trend upward, suggesting a return to the fasting level (average 0.825); unfortunately, the subjects were not observed long enough to demonstrate this point.

In the obese, non-diabetic group the respiratory quotients rose steadily from an average of 0.765 to 0.805 at the end of two hours dropping to 0.79 at the fourth hour (table 2). Only six of the group were observed through the eighth hour; then, the average quotients had returned to the average fasting level.

The average fasting quotient of the obese-diabetic group (table 3) was nearly the same as the non-diabetic group. The fat meal produced a slightly upward trend to 0.78 with a return to the fasting value in 5 hours. This curve was similar to the non-diabetic group, except that its rise was less and its fall came sooner.

The specific dynamic action of the fat meal. The total extra calories during absorption and assimilation of fat was essentially the same in the obese and normal groups, 25.25 and 26.15 calories, respectively, during a 5-hour period. The specific dynamic action based on surface area was, however, 30 per cent greater in normals. In the obese-diabetic group the total extra calories were 23 per cent higher than the average of the other groups. This effect of the meal in all groups was still evident at the end of the period of observation, even though the respiratory quotients had returned to the fasting levels in two of the groups, obese and obese-diabetics.

The specific dynamic action of a fat meal in the six individuals comprising the thin, normal sub-group was not significantly different from those of average weight, or those who

TABLE 2

Obese subjects

Subject	Age	Height in kg.	Height in cm.	Sex	B.M.R. per cent.	Basal Period			First Period Av. 90 min.			Second Period Av. 170 min.			Third Period Av. 260 min.			Fourth Period Av. 320 min.			Fifth Period Av. 400 min.		
						Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.
J.R.	40	105	158	F	111	279	0.77	39.2	315	0.77	44.5	320	0.79	45.1	326	0.81	46.1	315	0.80	44.2	320	0.84	45.6
M.S.	46	105	152	F	87	215	0.78	30.5	220	0.85	32.2	234	0.82	33.8	244	0.81	35.5	241	0.76	34.4			
S.J.	35	118	161	F	91	252	0.77	35.0	276	0.84	37.2	292	0.78	38.7	282	0.82	37.8	290	0.80	38.7			
H.P.	36	109	161	F	109	284	0.78	39.0	285	0.82	39.1	275	0.80	37.9	296	0.76	40.4	286	0.85	39.0	265	0.77	35.9
R.C.	18	105	166	F	95	261	0.75	35.4	295	0.84	40.7	259	0.82	35.8	277	0.83	38.4						
B.T.	45	100	153	F	97	252	0.74	33.8	261	0.79	36.5	269	0.80	39.8							276	0.74	40.1
E.S.	51	89	153	F	95	222	0.80	34.5	250	0.82	36.9	240	0.80	37.0	252	0.79	38.8	262	0.79	40.2	256	0.80	39.5
S.C.	51	106	166	F	107	275	0.75	36.9	296	0.78	40.2	298	0.78	40.5	300	0.74	40.3	315	0.72	41.8			
F.K.	17	107	172	F	97	275	0.83	36.4	285	0.76	36.9	297	0.77	38.6	275	0.80	35.8	280	0.76	36.4			
M.C.	17	97	164	F	90	248	0.75	33.3	248	0.82	33.8	254	0.80	34.6	257	0.78	34.7	255	0.79	34.3	249	0.80	33.8
E.J.	36	126	167	F	101	296	0.74	36.3	300	0.79	37.2	319	0.78	39.6							484	0.76	38.8
J.S.	37	93	157	F	100	256	0.82	36.0	267	0.82	40.1	259	0.77	38.3	256	0.78	38.0	269	0.78	39.9			
J.A.		156	151	F		335	0.77	38.9	350	0.81	38.8	361	0.74	41.6	398	0.76	45.1						
M.T.	35	102	170	F	91	250	0.72	33.1	261	0.76	35.0	281	0.79	37.9	254	0.78	34.1	264	0.79	35.6	265	0.79	35.8
L.G.	34	123	167	F	98	279	0.77	35.6	294	0.79	36.3	298	0.77	37.7	314	0.79	39.9						
H.R.	26	116	174	F	92	275	0.72	35.7	299	0.79	37.9				311	0.75	38.4						
L.O.	45	154	174	M	122	366	0.76	46.0	429	0.77	50.6	404	0.82	48.1	398	0.82	47.4	408	0.78	48.1	413	0.79	48.9
G.R.	26	86	151	F	96	235	0.72	36.0	256	0.76	36.8	248	0.85	39.1	251	0.76	36.8	241	0.77	37.3	254	0.79	39.5
F.D.	22	119	160	F	96	266	0.82	35.4	279	0.82	37.1	274	0.84	36.7	282	0.80	37.5	262	0.86	35.2			
C.J.	45	99	161	F	105	259	0.76	36.5	251	0.82	36.0	251	0.80	35.8	266	0.80	37.9	254	0.78	36.1			

TABLE 3

Obese diabetic patients

Subject	Age	Weight Kg.	Height Cm.	Sex	B.M.R. per cent.	Basal Period			First Period Av. 75 min.			Second Period Av. 145 min.			Third Period Av. 250 min.			Fourth Period Av. 325 min.			Fifth Period Av. 450 min.		
						Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.	Oz. consumption cc./min.	R.Q.	Cals./sq. m./hr.
M.W.	64	75	162	F	100	215	0.76	34.1	240	0.79	37.6	235	0.79	37.4	249	0.75	39.4	254	0.79	39.9			
C.S.	49	89	160	F	99	222	0.76	34.5	250	0.80	34.8	245	0.78	36.9	250	0.79	37.7	257	0.79	38.9			
M.M.	52	81	156	F	97	224	0.76	35.1	252	0.82	36.8	247	0.79	39.0	252	0.79	39.8						
A.K.	40	108	177	M	105	320	0.75	40.2	335	0.76	43.0	345	0.79	43.6	356	0.80	45.5						
M.D.	68	87	162	F	106	205	0.74	35.5	211	0.79	36.9	213	0.79	37.5	224	0.75	38.8	250	0.71	43.1			
J.M.	59	99	156	F	102	240	0.76	34.7	265	0.76	38.4	272	0.76	39.2	265	0.79	36.9	248	0.76	35.9			
W.R.	49	98	175	M	91	259	0.74	34.4	280	0.78	37.4	272	0.77	36.4	286	0.78	38.3	286	0.76	38.1			
L.B.	44	150	160	F	128	374	0.85	45.4	422	0.79	50.7	456	0.77	52.0				401	0.80	48.3	416	0.76	50.0
A.C.	60	84		F		218	0.76		239	0.75		262	0.81		253	0.80		251	0.72				
M.B.	40	82	160	F	96	216	0.79	33.7	240	0.76	37.5	239	0.76	37.0	256	0.81	40.2	279	0.72	42.6			
B.D.	35	83	154	F	76	175	0.75	27.5	200	0.76	31.6	201	0.77	32.0	196	0.76	31.0	192	0.79	30.5			

were obese. These individuals, however, although below the average weight, were not as thin as those studied by Mason ('27), upon whom he found an increased specific dynamic action of a fat.

The basal metabolic rate.. This was within the average accepted normal in all groups with these exceptions: L. B., an extremely obese diabetic who always showed an increased rate (four determinations on separate days). B. D., a diabetic, who had recently recovered from an infection, showed a low rate as would be expected. L. O., whose rate was plus 22 per cent, was a plethoric gourmand.

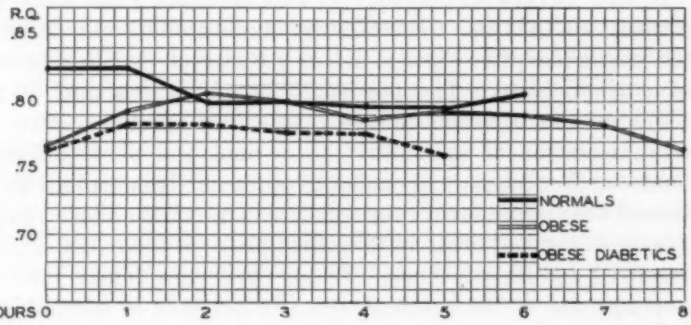

Fig. 1 Composite course of the respiratory quotient after fat meal; normal, obese, and obese diabetic individuals.

DISCUSSION

The most significant finding in this study is, we believe, that the post-absorptive respiratory quotient in the obese group was lower than that of the normal (fig. 1). Hagedorn ('27, '28) has reported the same result in a similar study of obese and normal subjects who had previously ingested a high carbohydrate diet. The quotients were higher than those obtained by us presumably because of this diet, but the average difference was the same—0.05. The explanation of this cannot readily be shown by direct observations, but it does permit of some attempt at analysis, as Hagedorn has done.

Lyon, Dunlop and Stewart ('32) also found the average of thirty-four observations on the respiratory quotient of obese subjects on a diet of 2500 calories (hospital maintenance diet) to be 0.755. The majority of the patients lost weight on it, so the authors considered it to be slightly sub-caloric.

Means ('15) published the metabolism data of eighteen obese subjects. Four of these he had studied himself, while fourteen were collected from the literature. The average post-absorptive respiratory quotient of his subjects was 0.755, whereas the others were 0.78.

The obese person may possess a mechanism by which he converts carbohydrate to fat with greater ease than the normal, so that the food mixture undergoing combustion in the post-absorptive state contains a relatively smaller amount of carbohydrate. The observations of Krantz and Means ('27) on the behavior of the respiratory quotient after the administration of epinephrin to normal and obese subjects, lends further support to such a hypothesis. It is ordinarily assumed that epinephrin causes a discharge of the more readily available carbohydrate in the expenditure of the extra calories required by its presence. These observers found that the average rise of the quotient was 19.6 per cent in normal individuals, whereas in the obese group it was 11.2 per cent. These observations were made when the subjects were in a post-absorptive state and it seems plausible that the failure of the quotient of the obese subjects to parallel the normal was because more of the carbohydrate, which had been previously ingested by the obese, had been converted to fat, rather than stored as such.

It is difficult to harmonize the above statements, however, with the fat-absorptive quotients that our experiments show. These would seem to indicate that both normal and obese use about the same food mixture after the ingestion of fat and imply that during this process at least the obese burns just as much carbohydrate as the normal. That there may be a carbohydrate-fat or fat-carbohydrate conversion at that stage, which is not reflected in the respiratory quotient, is unlikely,

unless it is compensated for. The more probable explanation would seem to be that fat requiring a certain amount of carbohydrate for its perfect combustion commands a release of carbohydrate from storage. It may be supposed that the obese diabetic's storehouse of carbohydrate is reduced, thereby accounting for the lesser rise after the ingestion of fat and, also, the more rapid fall to the base line. Our results are at variance with those of Wang, Strouse and Saunders ('25), who observed the effect for 5 hours of a fat meal on four obese subjects. The average fasting quotient in this group was 0.83 (non-protein) and the effect of the meal produced a downward trend of the quotient to 0.76 (average). Although the authors do not specifically state, it is assumed that these subjects had previously ingested an ordinary diet. The fat meal consisted of "fat varying from 55 to 126 grams in the form of slightly sweetened ice cream made from 40 per cent cream."

Attempts have been made by many investigators to explain obesity on the basis of a reduced specific dynamic action of food. This might be significant if the obese had little or no specific dynamic action in contrast to thin people. But, as Peters and Van Slyke ('31) point out, it could account for but 3 to 5 per cent of the total metabolism for the day. Evidence of a lowered specific dynamic action in the obese was largely based upon the work of Rolly ('21), Plaut ('23), and Wang, Strouse and Saunders ('24). More recent and extensive work by Lauter ('26), DuBois ('29), Strang and McClugage ('31), and Johnston ('32) throws a great doubt on there being any great difference in the specific dynamic action of food in the obese and normal person.

Although this study concerns the metabolism of fat only, no essential difference was found in the specific dynamic action in the obese and in the normal, when based upon total extra heat produced (fig. 2). For a 5-hour period the average obese spent 25.25 and the average normal 26.15 extra calories. So it appears that the total extra heat produced by fat absorption and assimilation is in both cases the same, but is distributed

over a greater cellular mass in the obese than in the normal. Strang and his co-workers ('31) observed a similar effect using a diet containg protein, 40 gm.; carbohydrate, 52 gm.; and fat, 26 gm. They believe that this extra heat produces a greater physiologic strain in the thin person, but does not necessarily represent a greater specific dynamic action as far as total calories are concerned.

In our normal group the absorptive quotients were depressed from the fasting level after the fat meal, while the opposite effect was found in the obese subjects. In the obese diabetics the absorptive quotients changed little, even though

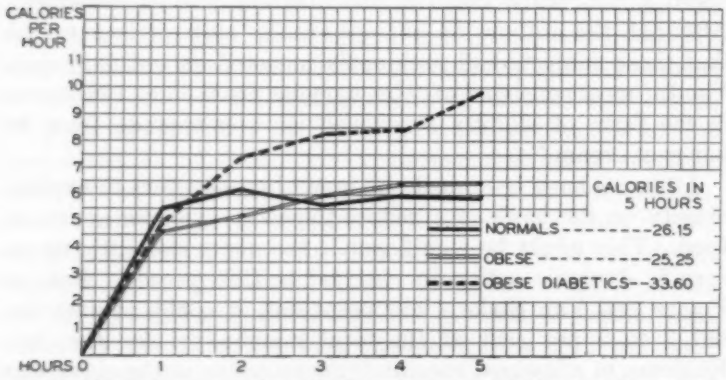

Fig. 2 Composite course of specific dynamic action (calories above basal) after fat meal; normal, obese, and obese diabetic individuals.

this group showed the greatest specific dynamic action. This is commented upon because of the work of Krogh and Lindhard ('20), who found that increase of metabolism caused by physical activity was accompanied by a lower quotient if the post-absorptive quotient was high, and a higher quotient if the post-absorptive quotient was low. Our results suggest that the same phenomena may take place if the increase in metabolism is caused by the specific dynamic action of fat. However, it will be seen that at the end of our observations the specific dynamic action was still present, although the respiratory quotients were returning to their respective fasting levels; this is particularly true in the obese diabetic subjects.

SUMMARY

1. The possibility that a qualitative metabolic variation exists in the obese person is discussed. This variation from the normal is based upon the finding of a lower post-absorptive respiratory quotient in the obese subject.

2. The effect of a large fat meal has been observed on twelve normal subjects, six of whom were slightly underweight, twenty non-diabetic obese and eleven obese diabetic subjects. The absorptive respiratory quotients indicate that the food mixture during this absorption is approximately the same in normal and obese persons. This response in the diabetic obese group suggests that less carbohydrate was being combusted during the utilization of fat. The observations were extended long enough to demonstrate the returning trend of the respiratory quotient to the post-absorptive level. No correlation between the curves of the absorptive respiratory quotients and the curves of the specific dynamic action could be demonstrated.

3. A specific dynamic action of fat was present in all groups. It was found to be approximately the same in the obese and the normal people. This action, however, was considerably greater in the obese diabetic individuals, which we have made no attempt to explain.

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CELLULOSE IN THE DIET OF RATS AND MICE

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TWO FIGURES

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Since the literature upon cellulose as a dietary constituent has been well reviewed recently by Rose ('32), we are including no review in this report. In a preliminary report (McCay, '29) we discussed the effect of feeding purified cellulose at very high levels to rats. Ordinary washed cellophane was used as a source of pure cellulose, since its physical composition is much more satisfactory for feeding animals than such substances as filter paper. Contrary to our expectations, rats fed diets containing 10 to 20 per cent cellulose were able to attain maturity, although the growth rate proved slower in these trials than that for animals in the colony fed a stock diet.

In order to determine the effect of this high cellulose [1] diet upon the life span of rats, we fed two groups upon the following diets: no. 4, casein 25, salt mixture 4, lard 20, sugar 36, cellulose 10, vitavose 5 and cod liver oil 3; no. 7, casein 25, salt mixture 4, lard 20, sugar 26, cellulose 20, vitavose 5 and cod liver oil 3. Eight male rats were placed upon diet 4 at

[1] In all cases in which we have employed pure cellulose in our diets it has been in the form of regenerated cellulose washed free from glycerine and ground finely. Most of this cellulose has been furnished through the courtesy of the Sylvania Corporation, New York City, and is the product marketed under the trade name of 'Sylphrap,' except that the compound fed has been washed free of glycerine. 'Regenerated cellulose' is the term commonly used in industry meaning cellulose purified by solution and reprecipitation.

the time of weaning. They attained a mean age of 674 ± 35 days. Nine male rats were fed diet 7 during the same period. They attained a mean age of 602 ± 61 days. For comparison we have shown in figure 1 the mortality curve for seventy-five male rats which were fed the stock diet used in our colony and previously described by Maynard ('30). The mean length of life for male rats upon this diet proved to be 503 ± 12. This stock diet has proved satisfactory for growth and reproduction for a number of years, but it is not satisfactory for longevity. The life span upon the lower cellulose level exceeds that found by Campbell ('28), upon her best diet. From

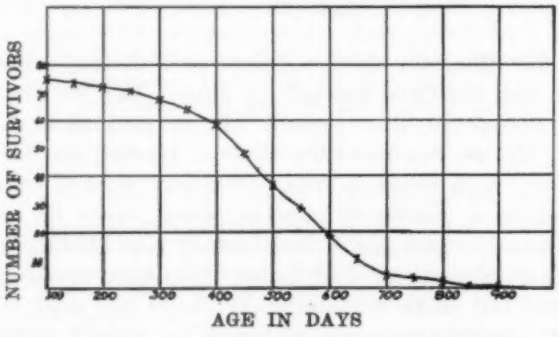

Fig. 1 Mortality curve for male rats fed a stock diet that allowed good growth and reproduction.

a consideration of the meager data of Slonaker ('12), it is evident that life spans of 600 odd days involve only middle life and that the life span for a rat must be well over 1000 days if we are to discuss it under the term of longevity in its usual meaning. There can be little doubt that such high cellulose diets are abnormal for Omnivora. The life span is not shortened, however. The longer life span in the present case of high cellulose diets probably has no relation to the cellulose, but is the result of the slow growth, as we have postulated elsewhere ('33).

In order to determine the influence of other inert materials upon the rate of growth the diets of table 1 were designed.

These diets all included a large amount of fat in order to determine whether the slower growth rates in the preceding experiments were due to the inability of the animal to ingest sufficient calories in the presence of large amounts of cellulose.

Table 2 contains the weights of rats at the start and after 12, 24 and 36 weeks for the animals upon each of these diets. In this case the better growth was made by the animals upon the diets containing the cellulose and agar, possibly indicating a better tolerance for the high calory diet in the presence of inert material. The growth rate was not improved over that previously found for diets 4 and 7, however. This indicated the animal body had ample capacity to ingest a considerable

TABLE 1

Diets for rats containing cellulose and agar-agar

NUMBER	8	9	10
Dry liver	25	25	25
Wheat germ	5	5	5
Cellulose	20	—	—
Agar-agar	—	20	—
Salt mixture	5	5	5
Sugar	—	—	20
Lard	40	40	40
Cod liver oil	5	5	5

TABLE 2

Mean weight and probable error of mean weight at start and after 12, 24 and 36 weeks feeding rats diets varying in inert materials

DIET NUMBER	NUMBER RATS AND SEX	MEAN WEIGHT, GM., BEGINNING	MEAN WEIGHT, GM., AFTER 12 WEEKS FEEDING	MEAN WEIGHT, GM., AFTER 24 WEEKS	MEAN WEIGHT, GM., AFTER 36 WEEKS	
8	4 ♂	43.0 ± 1.61	148.8 ± 10.29	205.3 ± 15.53	225.7 ± 11.70	High cellulose
8	6 ♀	38.8 ± 0.504	148.2 ± 5.81	223.3 ± 11.20	227.2 ± 13.60	High cellulose
Total	10 (♂ + ♀)	40.5 ± 0.80	148.4 ± 5.04	216.1 ± 8.86	226.7 ± 8.07	High cellulose
9	4 ♂	36.3 ± 2.50	145.8 ± 3.51	217.3 ± 8.59	275.0 ± 9.68	High agar
9	6 ♀	30.5 ± 2.00	139.3 ± 3.83	211.3 ± 9.50	240.3 ± 16.68	High agar
Total	10 (♂ + ♀)	32.8 ± 1.52	141.9 ± 2.65	213.7 ± 6.23	251.0 ± 10.97	High agar
10	4 ♂	47.0 ± 1.67	104.8 ± 9.19	187.5 ± 13.04	248.8 ± 6.81	No roughage
10	6 ♀	49.0 ± 1.00	88.0 ± 5.63	165.5 ± 6.70	220.8 ± 7.79	No roughage
Total	10 (♂ + ♀)	48.2 ± 0.86	94.7 ± 5.04	174.3 ± 6.60	232.0 ± 6.00	No roughage

excess of inert materials. These animals were maintained upon the diets for 9 months. They slowly attained a weight of 300 gm., but few exceeded it.

In these experiments no differences were observed between the agar and cellulose. The feces of all animals upon these diets were large, solid and light in color.

In the meantime, from many observations in the course of various experiments, the value of vitavose or wheat germ as a supplement to furnish adequate B factors had been doubted. For this reason the last experiments were repeated with new sources of vitamin B. The composition of these diets is shown in table 3.

TABLE 3

Second series of diets containing agar-agar and cellulose

NUMBER	27	8-A	9-A	10-A
Dry liver	25	25	25	25
Yeast	5	5	5	5
Cellulose	—	20	—	—
Agar-agar	—	—	20	—
Salt mixture	5	5	5	5
Sugar	45	—	—	20
Lard	15	40	40	40
Cod liver oil	5	5	5	5

These diets were designed to compare the growth rates of rats upon high roughage diets containing large amounts of fat with one without the roughage. Diet 27, containing a moderate amount of fat, was inserted, since earlier work had indicated a slower growth rate upon a high fat diet. In these series eight male rats were used for each diet. They were reared in the usual manner in cages with false bottoms.

The growth rates, summarized in table 4, upon diets 27, 8-a, 9-a and 10-a were the same. This indicates that in the presence of an adequate amount of yeast and with adequate calories the rat can consume enough food for optimum growth requirements even in the presence of large amounts of inert materials, such as cellulose or agar. The high intake levels of purified cellulose and agar-agar seem to give much the same results as far as growth and well being of animals can be observed from external appearances.

The rat can tolerate 20 per cent of inert material in its diet if the other ingredients furnish adequate vitamins. Optimum growth can be obtained with this high roughage diet. Earlier failures were due to inadequate vitamins.

The tolerance of mice for high roughage diets. Since it was found that rats could tolerate high levels of cellulose we desired to study another related species.

TABLE 4

Mean weights of male rats at the beginning and at 12 and 24 weeks of feeding diets with and without roughage. No differences in growth were found

DIET NUMBER	NUMBER RATS AND SEX	MEAN WEIGHT BEGINNING	MEAN WEIGHT AFTER 12 WEEKS FEEDING	MEAN WEIGHT AFTER 24 WEEKS	
		gm.	*gm.*	*gm.*	
8a	8 ♂	43.5 ± 1.59	198.4 ± 3.93	285.1 ± 4.33	High cellulose
9a	8 ♂	45.0 ± 2.26	190.6 ± 7.69	286.0 ± 8.94	High agar
10a	8 ♂	42.5 ± 1.98	200.5 ± 6.14	287.6 ± 5.97	No roughage
27	8 ♂	41.0 ± 2.12	195.4 ± 9.61	292.3 ± 8.09	No roughage

TABLE 5

Cellulose diets for mice

NUMBER	1-c	2-c	3-c	4-c
Dry liver	12	12	12	12
Casein	15	15	15	15
Lard	20	20	20	20
Starch	37	34	19	39
Yeast	5	5	5	5
Cod liver oil	5	5	5	5
Salt mixture	4	4	4	4
Cellulose	2	5	20	0

A year of preliminary studies feeding high roughage diets to white mice yielded consistent failures. These were due to inadequate supplements of B factors. Finally such supplements as vitavose were abandoned and yeast substituted. It was also found that cellulose must be ground very thoroughly to prevent selection of particles by white mice.

The diets shown in table 5 were used in the last series with mice.

Nine male mice were placed upon each of these diets shortly after weaning. The mean weights at the beginning of the experiment and again at the end of the tenth week are shown in table 6. Between the third and sixth weeks group 1-c and 4-c developed severe diarrhea and three of the animals in groups 1-c and 4-c died. Those upon the high cellulose diets seemed unaffected. These growth data for mice indicate they can grow quite normally upon diets containing large amounts of inert materials, such as cellulose. Other constituents in the diet must be sufficient for such growth, however.

Cellulose and intestinal injury. Although our earlier findings indicated rats can exist for about 600 days upon diets containing 20 per cent cellulose, there was the chance of internal injury of the gastro-intestinal tract until they slowly

TABLE 6

Weights of mice fed different roughage diets

DIET NUMBER	1-c	2-c	3-c	4-c
Beginning	6.5	6.5	7.5	7.4
10th week	21.6	20.0	20.6	23.4

adapted themselves to such diets. As a preliminary experiment to detect such injury we fed twenty-one rats for the 4 months following weaning upon a diet of casein 25, salt mixture 4, lard 17, sugar 26, yeast 5, cod liver oil 3 and cellulose 20. As a control we fed seven rats of the same age upon the same diet with the cellulose replaced by cooked starch. At the end of the period the rats were killed. The stomach and intestines were removed. Some hemorrhage was found in the intestines of all animals that had been upon the high cellulose diets with a slight hemorrhage in three of the seven controls. Histological examination showed some increase in the connective tissue and some degeneration in the tubular epithelium in four of the high cellulose cases and one of the controls.

To obtain more information concerning this intestinal irritation, we divided twenty-four rats, ranging in weight from

70 to 100 gm., into four groups. They were fed the diets of table 7 for 33 days.

These diets were designed to have approximately the same crude fiber content. In nos. 3-D and 4-D an attempt was also made to equalize the protein. Rice hulls were selected because of their reputation for inflicting intestinal injury.

TABLE 7

Diets used to study the intestinal irritation of rats by cellulose

DIET NUMBER	1-D	2-D	3-D	4-D
Yeast	2	2	2	2
Casein	25	25	24	19
Starch	47	47	33	11
Lard	10	10	10	10
Cod liver oil	5	5	5	5
Bone meal	1	1	1	1
Sodium chloride	1	1	1	1
Calcium carbonate	1	1	1	1
Bran	—	—	—	50
Rice hulls	—	—	23	—
Cellulose (fine)	—	8	—	—
Cellulose (coarse)	8	—	—	—
Mean gain in weight (gm.)	118	142	48	33

TABLE 8

Screen tests upon cellulose supplements

	PER CENT RETAIN IN		PASSED
	20 mesh per inch	40 mesh per inch	
Cellulose (fine)	0.0	0.9	99.1
Cellulose (coarse)	4.6	53.3	42.1
Rice hulls	0.0	20.2	79.8
Bran	0.0	64.2	35.8

The crude fiber (diet 2-D) was ground as finely as our facilities permit.

The screen tests upon these cellulose products are given in table 8.

Lighter colored and softer feces were obtained from animals upon rice hulls and bran. The most growth resulted from the finely ground cellulose diet with very poor growth upon the natural sources of roughage.

At the conclusion of the experiment the intestinal tracts of the animals were examined carefully. One animal upon the finely ground cellulose showed a marked irritation in the duodenum. Irritation was found in three of the rats upon the coarsely ground material. Signs of irritation were found in all animals upon both the bran and rice hull diets. In about half these latter cases the irritation was pronounced. The bran used was an ordinary commercial product such as that used in animal feeds and not a purified product. Cellulose produces irritation in certain rats from a given group.

TABLE 9

Composition of diets providing cellulose from various sources and the percentage of this cellulose digested by rats

DIET NUMBER	1-E	2-E	3-E	4-E	5-E	6-E
Casein	25.0	27.0	26.0	28.0	30.0	30.0
Salt mixture	5.0	5.0	5.0	5.0	5.0	5.0
Lard	12.5	12.5	12.5	12.5	12.5	12.5
Sugar	20.0	29.5	15.0	26.5	30.0	34.0
Cod liver oil	5.0	5.0	5.0	5.0	5.0	5.0
Yeast	5.0	5.0	5.0	5.0	5.0	5.0
Starch	5.0	5.0	5.0	5.0	5.0	5.0
Bran	22.5	11.0	—	—	—	—
Beet pulp	—	—	26.5	13.0	—	—
Cellulose	—	—	—	—	6.0	3.0
Per cent crude fiber in diet	6.0	3.0	6.0	3.0	6.0	3.0
Per cent crude fiber digested	24.3 ± 1.1	31 ± 1.1	41.8 ± 3.4	42.7 ± 2.4	17.4 ± 1	20.8 ± 0.9

The fineness of grinding is a factor in this irritation since the more finely ground material produces less irritation. In experiments with men that have not been published, we have found a marked relation between the coarseness of the cellulose and irritation at the time of excretion. These experiments show little difference between the irritation from rice hulls and cellulose.

The digestibility of cellulose from various sources and the fecal moisture. In order to compare the digestibility of cellulose from various sources, we employed the diets of table 9.

The method used in this study was to place six rats in separate metabolism cages. After a preliminary period of 3 days they were fed a charcoal marker. They were then fed diet 1-E for 2 weeks and the feces collected for the same time after the marker appeared. After feeding the second marker, the animals were shifted to diet 2-E. After a 3-day preliminary period they were fed a third marker and the feces collected from diet 2-E for 2 weeks. The other diets were run in turn upon the same animals. The crude fiber of the feed and feces was then determined by the procedure of Kohmoto ('26). The digestibility of these various celluloses is shown at the bottom of table 9. Nearly twice as much of the beet pulp fiber is digested as that of the other products. Regenerated cellulose is the least digested. Bran is intermediate. In the case of both bran and regenerated cellulose the 3 per cent level is digested slightly better than the 6 per cent one. Beet pulp cellulose is the most widely digested by rats.

We appreciate that the crude fiber determination probably measures many other substances than cellulose. There are also a number of different forms of cellulose that we are unable to distinguish to-day. The fact that beet pulp crude fiber seems more readily digestible than other forms probably means there are substances present in beet pulp that are analyzed as crude fiber, but that are quite different in digestibility. The solution of such problems as the relative digestibility of cellulose in plant products of different ages, such as lettuce or carrots, awaits the day of better analytical methods.

Cellulose and the weight of feces excreted. In order to determine the influence of cellulose upon the weight of feces excreted, we placed six adult rats in separate metabolism cages. A diet consisting of dried egg albumin 10, starch 47, lard 20, sugar 20, mineral mixture 3 was fed. Vitamin supplements of 100 mg. of Harris concentrate and a small allowance of the non-saponifiable fraction of cod liver oil were fed each day separately. The feed consumed and the dry weight of

the feces were determined for 6 weekly periods. Ten per cent of the starch was then replaced in the diet by cellulose and another series of collections was made for 6 weeks. The feed consumed and dry feces excreted for the six individuals for 6 weeks are shown in table 10. It is of interest to note that the dry weight of the feces increases beyond the amount that can be accounted for by the addition of the cellulose less its digestible portion.

TABLE 10

The effect of ingested cellulose upon the weight of rat feces

RAT NUMBER	FEED CONSUMED GRAMS PER RAT PER 6 WEEKS	FECES EXCRETED (DRY) GRAMS PER 6 WEEKS
	Cellulose	
1	663.0	132.6
2	457.0	94.7
3	509.9	110.8
4	488.5	99.5
5	475.1	97.3
6	593.0	123.6
Mean	531.1 ± 22.1	109.7 ± 4.3
	No cellulose	
1	442	41
2	479	41
3	427	38
4	309	25
5	508	47
6	482	42
Mean	441.2 ± 19.6	39.0 ± 2.0

During the 6 weeks of cellulose feeding the average rat ate 90 gm. of feed above that consumed without cellulose. But only 53 gm. of cellulose were ingested. If we assume that 17 per cent of this cellulose was digested, we would expect an increase of 44 gm. in the dry feces excreted. But the dry feces increased by an average of 70 gm. There may have been an average loss of 26 gm. of nutrients in addition to the cellulose loss in the feces in the course of 6 weeks.

We cannot say at this time whether this increase is due to the sweeping out of other nutrients by the cellulose or to increased cellular residues removed from the intestine. There

is certainly a marked difference in the fecal volume and weight with the ingestion of large amounts of cellulose.

Fecal moisture in relation to cellulose in the diet. Since fecal moisture is of real importance in the passage of feces, we ran a similar experiment to determine the moisture in feces when the cellulose of the diet originated from various sources. In figure 2, we have shown the moisture of these fresh feces. These determinations were made by placing the animal upon an experimental diet for 10 days. Five days after the beginning of the feeding, a 12-hour collection of the

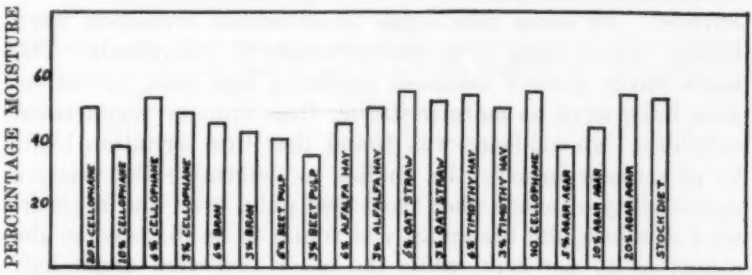

Fig. 2 Percentage moisture in the feces of rats in which the cellulose originated from various sources.

feces was made. They were placed in a tightly stoppered weighing bottle immediately after excretion. They were dried at 105° C. These data indicate that with the exception of agar the moisture per cent carried by the feces is not related to the content of inert material or its source. This is in conflict with the theory that the fecal moisture is governed by the cellulose. We realize that this cannot be a generalization, since we have studied only one species. Everyone appreciates the distinct properties of the feces of various species and that moisture is one of these characteristics.

SUMMARY

In preliminary experiments male rats fed 10 to 20 per cent cellulose diets were found to outlive the same sex fed a stock diet. This increase in the life span of these animals was

probably due to the slow rate at which they attained maturity rather than to a direct influence of the cellulose. However, it shows that the rat intestine is capable of tolerating these extreme levels of cellulose for a period in excess of that regarded as the normal life span. After a series of trials it proved possible to adjust the composition of the diet so that as good growth was obtained with diets containing 20 per cent cellulose or agar as with diets lacking roughage. Mice can also tolerate such levels of roughage and grow normally. In the course of an epidemic of diarrhea among the mice, it was observed that the animals on the high cellulose diet were not affected. In some rats signs of intestinal irritation were found. These seem to be characteristic of individuals. The more finely ground cellulose produced the least irritation. Rice hulls were no more irritating than bran or regenerated cellulose. No evidence was found that this irritation leads to permanent injury. In studies to determine the relative digestibility of crude fiber from beet pulp, bran and regenerated cellulose, the beet pulp was found to be digested to the extent of 40 per cent, while the other two were about half as well digested. In the case of bran and regeneratd cellulose 3 per cent levels are slightly better digested than 6 per cent ones. In a determination of the effect of the cellulose ingested upon the feces excreted it was found that the dry weight of these feces increases beyond an amount that can be ascribed to the ingested cellulose. Either there is a decreased digestibility of nutrients or the cellulose causes increased losses in the feces of such materials as epithelial cells from the intestine. No relation could be established between the moisture content of the feces and the level of roughage ingested, except in the case of agar. In the case of this material there was a direct relationship.

We wish to thank Prof. L. A. Maynard for many suggestions in the course of these experiments.

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A COMPARISON OF VITAMINS B AND G IN CANNED STRAINED FOODS [1]

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The recognition of several factors in the undifferentiated vitamin B required for rat growth has necessitated repetition of earlier assays. Since the antineuritic vitamin B is susceptible to destruction by moist heat, we might expect canned foods to vary in vitamin B content and its ratio to vitamin G potency, depending upon the heat process used in the canning method. Tests performed on canned strained foods give a measure of their value as sources of vitamins in the form in which they are widely used for infant and convalescent diets. Therefore, we have determined the vitamin B and G potency and thus the ratio of these two factors in some canned strained foods: tomatoes, carrots, peas, green beans, beets, spinach, a vegetable soup, and a canned cereal.

EXPERIMENTAL

The determinations of the vitamin B and G content were made upon rats raised in this laboratory, 28 to 29 days old and weighing 35 to 60 gm. They were kept in individual cages with raised screen bottoms, 3 meshes to the inch. In order to control coprophagy more closely, we adopted the use of rat 'harnesses' as described by Page ('32) for the tests of both vitamins. In other respects, the usual technic for conducting an animal test was observed as described in a previous paper (Hanning, '33).

[1] Financed by a grant from the Gerber Products Company.

The vitamin B deficient diet was that of Chase and Sherman ('31), except that the casein was extracted with dilute acetic acid, and the vitamin G deficient diet that of Bourquin and Sherman ('31). While these diets may not be entirely adequate in regard to other B vitamins required by the rat, it was felt that with the short test period of 5 weeks and the natural foodstuffs being tested, there probably was no significant deficiency of any except the one vitamin being determined.

After a preliminary depletion period averaging 16.4 days on the B deficient diet and 13.8 days on the G deficient diet, the supplemental feedings were begun and continued 5 weeks. Except for the rats fed the B deficient diet plus canned strained spinach and to some extent, beets, there was no great difficulty in obtaining complete consumption of the supplements. The data of all rats not consuming the required amount were discarded.

The supplements used throughout were furnished us by the Gerber Products Company, the canning method being briefly described in a previous paper ('33). The tomatoes, carrots, green beans and beets were representative samples of the entire 1932 season pack. Spinach no. 3 was a similarly representative sample of spring spinach of 1932, while spinach no. 1 was from 1 day's pack of fall spinach canned in 1931. Peas no. 1 was of a variety known as Perfection and no. 2 Thomas Laxton, both canned in 1931 from 1 day's pack. The vegetable soup and the canned cereal were taken from the warehouse as representing the usual product of 1932.

RESULTS

I. Vitamin B content of the vegetables

The data of vitamin B tests of the vegetables are presented in table 1. The potencies are expressed in terms of units as that amount which when fed daily produced an average gain of 3 gm. per week. The data indicate that 2 gm. of canned strained tomatoes or 3 gm. of strained peas, either no. 1 or no. 2, with somewhat different total solids content, gave more than unit growth.

On the other hand, it required 7 gm. of canned strained carrots for 1 unit, but 10 gm. of strained green beans or beets contained more than 1 unit of vitamin B. Because of the difficulty of obtaining complete consumption, the largest amount of spinach fed was 6 gm. Receiving this quantity, the rats lost in weight to approximately the same extent as those fed 5 gm. of strained green beans, which amount contained

TABLE 1

Record of rats fed canned strained vegetables as sources of vitamin B

AMOUNT OF CANNED STRAINED FOOD FED DAILY[1]	NUMBER OF RATS	WEIGHT AT DEPLETION[2]	DAILY FOOD CONSUMPTION[2]	AVERAGE WEEKLY GAIN[2]	UNITS OF VITAMIN B PER OUNCE
		gm.	*gm.*	*gm.*	
1 gm. tomatoes	4	66.75	4.04	+ 0.30	
2 gm. tomatoes	6	68.67	5.34	+ 4.75	24.0
3 gm. peas no. 1	8	64.75	4.01	+ 3.55	
4 gm. peas no. 1	7	63.71	4.56	+ 4.94	
3 gm. peas no. 2	8	65.00	4.14	+ 3.80	7.0–8.0
5 gm. carrots	5	63.20	4.09	+ 1.44	
7 gm. carrots	6	68.67	4.83	+ 3.03	
10 gm. carrots	6	67.16	5.29	+ 6.80	4.3
4 gm. green beans	6	59.83	4.99	— 4.03	
5 gm. green beans	8	58.63	4.05	— 0.73	
10 gm. green beans	7	68.00	5.22	+ 4.69	4.7
7 gm. beets	4	69.75	3.96	— 1.20	
10 gm. beets	5	68.20	4.18	+ 3.44	3.3
4 gm. spinach no. 3	8	63.63	4.26	— 4.75	
6 gm. spinach no. 3	4	64.60	4.34	— 1.32	2.5
None	33	64.76	2.95[3]	— 6.93[3]	

[1] Of the representative samples, 1 gm. of canned strained tomatoes was produced from 2.65 gm. raw; of strained carrots, from 0.65 gm. raw; of strained green beans, 0.64 gm. raw; of strained beets, from 0.85 gm. raw; and 1 gm. canned strained spinach (no. 3) was produced from 1 gm. raw spinach. Although the peas were not representative of the entire season's pack, 1 gm. of the strained product was approximately equivalent to 0.73 gm. raw peas.

[2] Averages for the group.

[3] All except three of the rats died before the end of the test—the average period being 22.8 days. Averages are for the survival instead of the entire test period.

½ unit of vitamin B. Therefore, 12 gm. of strained spinach probably contained 1 unit. These potencies are expressed also in terms of units per ounce in the last column of table 1.

II. Vitamin G content of the vegetables

The record of the vitamin G tests of the vegetables is presented in table 2. Here, again, the unit is defined as the

TABLE 2

Record of rats fed canned strained vegetables as sources of vitamin G

AMOUNT OF CANNED STRAINED FOOD FED DAILY[1]	NUMBER OF RATS	WEIGHT AT DEPLETION[2]	DAILY FOOD CONSUMPTION[2]	AVERAGE WEEKLY GAIN[2]	UNITS OF VITAMIN G PER OUNCE
		gm.	*gm.*	*gm.*	
3 gm. spinach no. 1	8	53.00	5.40	+ 4.05	
1 gm. spinach no. 3	7	50.70	4.38	— 0.40	
3 gm. spinach no. 3	8	51.38	4.92	+ 2.28	
5 gm. spinach no. 3	7	50.38	6.07	+ 4.80	7.5–11.8
3 gm. peas no. 1	7	45.43	4.21	+ 2.69	
5 gm. peas no. 1	14	51.72	4.91	+ 5.50	
7 gm. peas no. 1	6	45.17	4.60	+ 6.20	
5 gm. peas no. 2	12	52.67	5.20	+ 4.37	7.5– 8.6
4 gm. tomatoes	7	49.85	5.10	+ 2.43	
5 gm. tomatoes	5	52.60	5.06	+ 3.72	4.0– 5.0
3 gm. green beans	6	55.00	5.23	+ 1.07	
5 gm. green beans	7	52.57	5.02	+ 2.94	6.0
7 gm. beats	7	50.67	4.49	+ 2.23	
10 gm. beets	5	49.00	4.81	+ 3.56	3.3
7 gm. carrots	8	49.75	3.90	+ 0.95	
10 gm. carrots	7	47.67	4.04	+ 2.43	2.5
None	28[3]	51.86	4.11	— 0.91	

[1] Of the representative samples, 1 gm. of canned strained tomatoes was produced from 2.65 gm. raw; of strained green peas, 0.64 gm. raw; of strained beets from 0.85 gm. raw; of strained carrots, 0.65 gm. raw; and 1 gm. canned strained spinach no. 3 was produced from 1 gm. raw spinach. Spinach no. 1 was not a representative sample. Although the peas were not representative of the entire season's pack, 1 gm. of the strained product was approximately equivalent to 0.73 gm. raw peas.

[2] Average for the group.

[3] One rat died 3 days before the end of the test period; the others survived the entire period.

amount which when fed daily produced a 3-gm. gain per week. The strained peas and spinach were very good sources of vitamin G but the two samples of each were different in potency. Of spinach no. 1 containing 6.78 per cent total solids, 3 gm. gave more than unit growth; of no. 3 with 6.30 per cent total solids, less. Peas no. 1 gave better growth than no. 2. This can probably be explained by the higher total solid content, 12.33 per cent in no. 1, while no. 2 contained 10.83 per cent total solids. Thus, 3 to 4 gm. of canned strained spinach or peas contain about 1 unit of vitamin G. Approximately unit growth was obtained upon 4 to 5 gm. strained tomatoes, 5 gm. green beans, or 10 gm. of strained beets. Of strained carrots, 10 gm. gave less than unit growth. The values calculated to units per ounce are also listed.

III. Tests of cereal and vegetable soup

In comparison with the data on vegetables, our tests of the canned strained cereal indicate that 4 gm. contain 1 unit vitamin B and 2.5 gm., 1 unit vitamin G. The large quantity of milk used in its manufacture is responsible for the high vitamin G potency. The vegetable soup contained equal amounts of the B and G vitamins, 10 gm. being required for 1 unit of each.

DISCUSSION

It is interesting to note that the canned strained tomatoes contain, according to our tests, about three times as many units of vitamin B as of vitamin G. This is indicated by comparing the values as expressed in units per ounce. The strained carrots and peas contain about one and a half times as many units of vitamin B as vitamin G, while beets have equal amounts of each vitamin. On the other hand, the canned strained green beans contain twice and spinach about three to four times as many units of vitamin G as vitamin B. Thus, in the canned strained tomatoes, carrots, and peas, vitamin B predominates; in spinach and green beans, vitamin G, but equal amounts of each vitamin are found in beets.

An exhaustive study of differentiated vitamin B determinations on rats has been made by Roscoe in England ('30, '31 a, '31 b) who expressed the potency in terms of that of dried brewer's yeast. The amount of yeast (0.05 gm. for B and 0.1 gm. for G) which would induce 50 to 60 gm. gain in 5 weeks was taken as 100. On that basis she rated spinach and carrots as 5 to 10 and tomatoes as 2.5 to 5 in vitamin B; in vitamin G, spinach as 10 to 20, carrots 5 to 10 and tomatoes 2.5 to 5. Since it requires twice as much yeast for her standard of potency of 100 in vitamin G as in vitamin B, spinach would have about four times as much vitamin G as B, if rated on growth as in our own data. In the same manner, carrots and tomatoes would have twice as much vitamin G as vitamin B. In our work upon canned strained foods, as noted above, we found three to four times as much vitamin G as vitamin B in spinach, but three times as much vitamin B as vitamin G in tomatoes, and one and a half times more vitamin B than vitamin G in carrots. That is, in the two studies, the ratio is the same for spinach, but our samples of carrots and tomatoes contained a much greater proportion of vitamin B to vitamin G.

In this country, the work of Rogers ('28) indicates a greater concentration of vitamin G than vitamin B in spinach and green beans whether raw or cooked or canned. Jones and Nelson ('30) fed juice from canned vine-ripened tomatoes. From the growth curves presented, it would appear that 5 cc. of the juice contained much more than 1 unit vitamin B in terms of the 3 gm. gain per week standard. Hartley is quoted by Sherman ('31) as having determined the vitamin G in the juice from canned tomatoes to be 0.16 units per gram. Day ('31) studied the vitamin G content of a number of vegetables feeding the raw food. He lists carrots and beets as containing 0.5 unit and winter spinach 1 unit per gram, based on an 8 weeks' test.

We realize, however, that it is difficult to make direct comparisons as to the vitamin B and G potency of the various foods because of varying factors in the experimental method.

This is especially true of the control of coprophagy by 'rat harnesses.' We have found in this laboratory that their use results in much lower but more reliable and concordant data for both vitamins B and G. How far these tests are influenced by a lack of vitamin B_4 or other B factors cannot be surmised.

SUMMARY

From data collected under very carefully controlled conditions with the use of rat 'harnesses' to prevent coprophagy, it was found that 1 ounce of canned strained tomatoes contained from 20 to 24 units vitamin B and 6.7 units vitamin G; of canned strained peas, 7 to 8 units vitamin B and 7.5 to 8.6 units vitamin G; of canned strained carrots 4.3 units vitamin B and 2.5 units vitamin G. The canned strained beets contained 3.3 units of each vitamin, and the canned strained green beans contained 4.7 units vitamins B, and 6 units vitamin G. The canned strained spinach was low in vitamin B, containing probably 2.5 units, with 7.5 to 11.8 units of vitamin G per ounce.

In three of the canned strained vegetables: tomatoes, peas and carrots, there was more vitamin B than vitamin G; in green beans and spinach, more vitamin G than vitamin B; and in canned strained beets, equal amounts of each vitamin.

A canned strained cereal contained 7.5 units vitamin B, and 12 units vitamin G per ounce. In a canned vegetable soup there were 3 units of each vitamin per ounce.

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INANITION AS A FACTOR IN VITAMIN G DEFICIENCY

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Since the experiments of Smith and Hendrick ('26) and Goldberger and Lillie ('26) it has been known that animals restricted to a vitamin G deficient diet become stunted and frequently develop a characteristic dermatitis. The skin lesions are undoubtedly a manifestation of the specific lack of vitamin G. The failure to grow and the accompanying changes in the tissues and organs of the body have been tacitly attributed to the vitamin deficiency also. Little attention has been directed to the food intake of the animals. While the food consumption in vitamin G deficiency has been recognized as subnormal, yet, in comparison with the anorexia of vitamin B deficiency, it is sufficiently high to have led to the conclusion that vitamin G plays a negligible part in the maintenance of the appetite. It remained for Graham and Griffith ('33) to emphasize the importance of vitamin G as an extrinsic factor in the physiological mechanism controlling the consumption of food. The present experiments were planned to study the effects of vitamin G deficiency, as opposed to simple caloric insufficiency, on some of the tissues of the body. The mouse was selected as the experimental animal, following the work of Bing and Mendel ('29).

EXPERIMENTS

Three-week-old mice were removed from their mothers and divided into three groups. All animals were fed a basal diet

plus 2 drops of cod liver oil daily. The 'normal' animals received 250 mg. of dried yeast per day and the mice in the 'G-deficient' group received 0.1 cc. of a vitamin B containing sirup. The 'controls' received the same amount of yeast as did normal animals, but the total food intake was restricted to the amount consumed by the G-deficient mice.

The basal diet was prepared by mixing 200 gm. of extracted casein, 265 gm. of cornstarch, 200 gm. of lard and 40 gm. of the McCollum salt mixture. The vitamin B preparation was made by extracting 2 kg. of wheat germ (which contained 5.6 per cent moisture) with 5 liters of ordinary alcohol. The filtrate from the extracted wheat germ was evaporated in vacuo at a temperature not exceeding 60° until all the alcohol was removed. The residue was diluted with two volumes of water, placed in the refrigerator overnight, and then filtered while cold. The product consisted of a sirupy solution which contained 25 per cent solids and 0.2 per cent ash. Assay of the solution showed that 0.1 cc. provided sufficient vitamin B to meet the growth requirements of mice.

We have found that animals fed upon the G-deficient diet fail to grow and die in an average time of 7 weeks. About 75 per cent of the animals develop an ulcerative dermatitis, usually in the axillary region or beneath the lower jaw. No signs of changes in the eyeball have been observed macroscopically. The addition of small amounts of crystalline carotene, from 0.001 mg. to 0.100 mg. per day has no influence on the survival time or on the incidence of dermatitis.

In the present study the experimental period was limited to 6 weeks so as to avoid the factor of complete anorexia which characterizes the terminal stages of vitamin G deficiency. At the end of this time, the mice were sacrificed and their tissues were examined chemically. The hemoglobin concentration of the blood and the number of red cells were determined by the method of Heinle and Bing ('33). The serum protein concentration was estimated by digestion of 0.025 cc. of blood serum with sulfuric acid and 30 per cent H_2O_2, followed by direct Nesslerization essentially as described by Myers ('24).

The femurs were removed and analyzed by the methods employed by Karelitz and Shohl ('27). The fat was extracted by the Bloor method ('26) from the entire carcasses of mice after the removal of the stomach and intestines. This procedure yields a solution of the lipids in petroleum ether. The total fat was estimated by evaporating the solvent and weighing the residue. Phospholipids were determined by analysis of an aliquot portion of the petroleum ether solution for phosphorus by the method of Kuttner and Lichtenstein ('32).

RESULTS AND CONCLUSIONS

The average values of the figures obtained on individual mice are presented in tables 1 and 2.

Growth and food consumption. The calorie-deficient and G-deficient animals were stunted in weight. The total average food intakes of the stunted mice were approximately 60 per cent of normal.

Since the early work of Karr ('20) and Cowgill ('21) the vitamin B complex has been recognized as a dietary factor that exerts a specific effect on the appetite. Burack and Cowgill ('31) showed that dogs deprived of the vitamin B complex, until they no longer consumed their food, had their appetites restored by administration of vitamin B but not by vitamin G. Our data show that vitamin B evokes a maintenance appetite for a limited time, probably until the animal's available store of vitamin G is depleted, when the appetite fails.

Composition of the blood. The average values of the hemoglobin concentration and the number of red cells were slightly below normal in both G-deficient and calorie-deficient mice, the cells being less affected than the hemoglobin. Sure, Kik and Smith ('31) and Guha and Mapson ('31) have reported a somewhat more marked decrease in the hemoglobin and red blood cells of rats maintained on a G-deficient diet. Normal mice at the age of 3 weeks have about 8.0 gm. of hemoglobin per 100 cc. of blood, which is less than the hemoglobin concentration of the stunted animals.

The serum protein concentration in the calorie restricted mice was less than that of the G-deficient animals, but, because of the small number of observations, further experiments on this point would be desirable.

Composition of the femurs. The figures in table 1 show that the stunted mice had lighter bones with less ash than normal animals. In 3-week-old mice weighing 9.5 gm., the femur weighs 10.5 mg. when dried, the ash content averages 4.5 mg., and the percentage of ash in the dried and defatted bone is 44.3 per cent. It is therefore evident that in stunted

TABLE 1

Average composition of blood and bones in vitamin G deficiency

	NORMAL	CONTROL	G-DEFICIENT
Number of mice	7	7	7
Initial weight	10.8 gm.	10.4 gm.	10.0 gm.
Final weight	21.9 gm.	9.8 gm.	9.7 gm.
Food intake	98.1 gm.	54.6 gm.	55.7 gm.
Hemoglobin	12.77 gm.	10.27 gm.	11.18 gm.
Red blood cells	9.83 million	9.06 million	9.60 million
Serum proteins	5.36 gm.	3.95 gm.	5.04 gm.
Weight dried femur	33.2 mg.	20.3 mg.	19.7 mg.
Extracted weight	31.6 mg.	19.7 mg.	18.4 mg.
Ash weight	18.8 mg.	10.1 mg.	9.5 mg.
Per cent ash	59.3 per cent	50.9 per cent	51.8 per cent

growth, produced by either calorie or vitamin G restriction, the long bones continue to grow at a sub-normal rate. The dried weight of the femurs is almost doubled and the weight of the ash is more than doubled in 6 weeks, even though the body weight remains practically constant. The phenomenon of persistent skeletal growth in stunted rats has been demonstrated by Winters, Smith and Mendel ('27).

Body fat and total phospholipid content. The data presented in table 2 show a very close resemblance in the amount of fat and phospholipid in the two groups of stunted mice. For comparison, five normal mice were sacrificed at the age of 3 weeks and analyzed. The average values were as follows:

live weight, 9.5 gm.; net body weight, 8.9 gm.; ether extract, 1.13 gm.; lipid phosphorus, 5.34 mg. These figures indicate that the fat content is not only very low in the stunted mice, but it is actually much less than the animals presumably have at the beginning of the experiment. It is of interest that the phospholipid content of the body remains relatively constant in mice that are stunted, even though the neutral fat practically disappears. This may be considered as further evidence of the importance of phospholipids as essential components of the tissues, in accordance with the conception of the élémente constant of Terroine and Belin ('27).

TABLE 2

Average content of body fat and phospholipids in vitamin G deficiency

	NORMAL	CONTROL	G-DEFICIENT
Number of mice	6	7	7
Initial weight	9.3 gm.	9.7 gm.	9.3 gm.
Final weight	20.9 gm.	10.2 gm.	9.7 gm.
Food intake	94.9 gm.	63.9 gm.	63.5 gm.
Net body weight	18.0 gm.	8.8 gm.	7.8 gm.
Ether extract	2.86 gm.	0.48 gm.	0.46 gm.
Lipid P	7.94 mg.	5.79 mg.	5.78 mg.

SUMMARY

Young mice, receiving a vitamin G deficient diet for 6 weeks, consume approximately 60 per cent of the caloric intake of other mice fed a complete diet. In both calorie-deficient and G-deficient mice the body weight remains approximately constant, the neutral fat almost disappears from the body, the total phospholipid content remains unchanged, and the bones continue to grow at a sub-normal rate. The number of red blood cells and the concentration of hemoglobin and of serum protein are slightly lower in stunted mice than in normal animals of the same age. Inanition is thus a significant feature of the syndrome of vitamin G deficiency. Both vitamins G and B are required for the maintenance of the normal appetite.

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CAROTENE AND VITAMIN A REQUIREMENTS FOR WHITE LEGHORN CHICKS

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FOUR TEXT FIGURES AND ONE PLATE

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The object of this study was to obtain data regarding the minimum requirements of carotene and vitamin A for White Leghorn chicks, and to determine whether such data might have an application in a quantitative test for vitamin A.

The battery brooder used was the conventional type with electric heating units and thermostatic control. For each compartment containing a heating unit there was a duplicate compartment without a heating unit. A removable partition between the two compartments made it possible to provide additional space for each group as required. Food and water troughs were provided in the usual manner. The brooder was located in a well-insulated room ventilated only by windows and protected from direct sunlight. Before using the brooder, it was taken apart, scrubbed with soap and water and steamed.

EXPERIMENTAL

In order to obtain the desired information, we divided 612 day-old White Leghorn chicks into the following groups:

Group A, composed of ten groups of ten chicks each. The chicks in these groups received graduated doses of carotene in oil of known potency (sample A) for 30 days in addition to the basal diet which they received throughout the test period.

The doses used were decimal graduations from 10 to 100 A.D.M.A. units.

Group B, composed of ten groups of ten chicks each. The chicks in these groups received graduated doses of carotene in oil of unknown potency (sample B) for 30 days in addition to the basal diet which they received throughout the test period. The doses used were decimal graduations from 10 to 100 A.D.M.A. units.

Group C, composed of nine groups of ten chicks each. The chicks in these groups received graduated doses of carotene in oil of unknown potency (sample C) for 30 days in addition to the basal diet which they received throughout the test period. The doses used were decimal graduations from 10 to 90 A.D.M.A. units.

Group D, composed of nine groups of ten chicks each. The chicks in these groups received graduated doses of cod liver oil of known potency (sample D) for 30 days in addition to the basal diet which they received throughout the test period. The doses used were decimal graduations from 10 to 90 A.D.M.A. units.

The control group contained 232 chicks, which received the basal diet only.

Beginning with the first day, all groups received the vitamin A-free basal diet, which consisted of:

52½ pounds white corn, ground
10 pounds wheat bran
15 pounds wheat middlings
10 pounds meat scrap
10 pounds skimmed milk powder
2 pounds calcium carbonate
½ pound sodium chloride
100 A.D.M.A. units of vitamin D per chick per day.

The carotene and vitamin A solutions were fed daily by mouth with standardized medicine droppers for 30 days starting with June 17, 1933. These solutions were diluted with refined cottonseed oil so that one drop carried the particular graduated dose of carotene or vitamin A plus 100 A.D.M.A. units of vitamin D. The control group received no carotene or vitamin A, but was given 100 units of vitamin D in one drop of the same oil used for diluting. All chicks received 100 Sherman units of vitamin D per day throughout the test period. The source of vitamin D was a vitamin D concentrate

from cod liver oil produced by the Zucker process (Columbia University). The relatively small amount of vitamin A normally present in this concentrate was destroyed by oxidizing at 100°C. with a current of air for 24 hours.

Inasmuch as the control group received no carotene or vitamin A throughout the experimental period and whereas the members of groups A, B, C, and D received various amounts of carotene or vitamin A during a period of 30 days, the control group became depleted in carotene and vitamin A much sooner than the other groups. If we assume that there exists a certain minimum number of units of vitamin A or carotene, *X*, which if supplied daily to each chick will permit it to live a normal life, then it is evident that the carotene or vitamin A supplied to the members of groups A, B, C, and D should permit them to live a certain number of days longer than the members of the control group. The average number of days of additional life and the number of units of carotene or vitamin A required by the animals of groups A, B, C, and D, respectively, to live an extra day over the average number of days lived by the animals of the control group was calculated and is presented with a summary of the experimental results in table 1.

The 232 controls which received no carotene or vitamin A supplement lived a grand total of 6310 days; an average of 27.2 days per chick.

In group A, in which carotene in oil of known potency was given, the average of ninety chicks showed that the group lived 1 additional day for each 114 A.D.M.A. units of vitamin A received. Since there were only ninety chicks in the C and D groups, the averages of the corresponding ninety chicks in the A and B groups were used. If, however, the total of 100 chicks was used, the number of A.D.M.A. units required to live 1 extra day was 116, as compared to the 114 with the ninety chicks.

Group B was given carotene in oil of unknown potency, but it was fed on the basis of having the same potency as the A group. On this basis, it took 160 A.D.M.A. units per chick

TABLE 1

Summary of results. Carotene and vitamin A requirement experiments with 612 day-old White Leghorn chicks

	NUMBER OF VITAMIN A UNITS EACH CHICK RECEIVED DAILY	10	20	30	40	50	60	70	80	90	100	AVERAGE OF 100 CHICKS	AVERAGE OF 90 CHICKS
Group A, carotene in oil known potency (sample A)	Chicks in group	9	10	10	10	10	10	9	10	10	10	98	88
	Units group received	2630	5380	8460	10000	13700	17280	18900	21920	24030	30000	152300	122300
	Days group lived	302	341	407	360	408	407	398	422	403	517	3955	3468
	Units per chick	292	538	846	1000	1370	1728	2100	2192	2403	3000	1554	1390
	Average life per chick	33.6	34.1	40.7	36.0	40.8	40.7	44.2	44.2	40.3	51.7	40.6	39.4
	Extra days lived per chick	6.4	6.9	13.5	8.8	13.6	13.5	17.0	17.0	13.1	24.5	13.4	12.2
	Units required to live extra day	46	78	63	114	101	128	124	129	183	122	116	114
	Per cent potency, carotene of known potency											100	100
Group B, carotene in oil unknown potency (sample B)	Chicks in group	10	10	10	10	10	10	10	10	10	10	100	90
	Units group received	2550	5960	8010	10760	13800	17040	18690	20560	23850	27000	148220	121220
	Days group lived	295	382	308	360	372	401	376	355	356	355	3560	3205
	Units per chick	255	596	801	1076	1380	1704	1869	2056	2385	2700	1482	1347
	Average life per chick	29.5	38.2	30.8	36.0	37.2	40.1	37.6	35.5	35.6	35.5	35.6	35.6
	Extra days lived per chick	2.3	11.0	3.6	8.8	10.0	12.9	10.4	8.3	8.4	8.3	8.4	8.4
	Units required to live extra day	111	54	223	122	138	132	180	248	284	325	176	160
	Per cent potency of carotene of A											65.9	71.3
Group C, carotene in oil unknown potency (sample C)	Chicks in group	10	10	9	10	10	10	10	10	10			89
	Units group received	2790	4840	7050	10960	13950	15900	21000	23280	25290			125060
	Days group lived	326	272	289	344	325	342	413	386	386			3083
	Units per chick	279	484	783	1096	1395	1590	2100	2328	2529			1405
	Average life per chick	32.6	27.2	32.1	34.4	32.5	34.2	41.3	38.6	38.6			34.6
	Extra days lived per chick	5.4	0.0	4.9	7.2	5.3	7.0	14.1	11.4	11.4			7.4
	Units required to live extra day	52	.....	160	152	263	227	149	204	222			190
	Per cent potency of carotene of A												60.0
Group D, cod liver oil (sample D)	Chicks in group	10	9	10	10	10	10	10	10	10			89
	Units group received	2690	5220	9000	11360	14900	16740	20370	20320	24840			125440
	Days group lived	311	328	394	380	409	377	422	360	416			3397
	Units per chick	269	580	900	1136	1490	1674	2037	2032	2484			1409
	Average life per chick	31.1	36.4	39.2	38.0	40.9	37.7	42.2	36.0	41.6			38.2
	Extra days lived per chick	3.9	9.2	12.0	10.8	13.7	10.5	15.0	8.8	14.4			11.0
	Units required to live extra day	69	63	75	105	109	159	136	231	173			128
	Per cent potency of carotene of A												89.1
Control group, basal ration	Chicks in group												232
	Units group received												none
	Days group lived												6310
	Average life per chick												27.2

to live an extra day, indicating this solution of carotene in oil to be 71.3 per cent of the potency of the carotene in oil fed in group A.

Group C given carotene in oil of unknown potency was fed on the basis of having the same potency as A, and showed a requirement of 190 A.D.M.A. units to live 1 extra day, indicating this carotene in oil to be 60 per cent of the potency of the carotene in oil fed in group A.

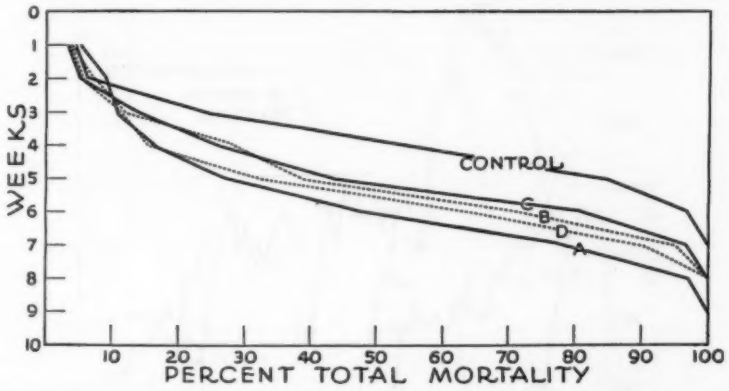

Fig. 1 Mortality curve. Six hundred and twelve White Leghorn chicks. Summary of results. Carotene and vitamin A requirement experiments with 612 day-old White Leghorn chicks. Group A—Carotene in oil—known potency (sample A). Group B—Carotene in oil—unknown potency (sample B). Group C—Carotene in oil—unknown potency (sample C). Group D—Cod liver oil (sample D). Control group, basal ration.

In group D, biologically tested cod liver oil was used in amounts equivalent to that of the carotene in oil of known potency fed to group A. The figure obtained for this group should, therefore, be the same as that of group A. Instead it was 87.9 per cent of the potency of carotene in oil fed in group A. The results were 128 A.D.M.A. units for each extra day, as compared to 114 A.D.M.A. units, or within 10.9 per cent of the expected results.

Figure 1 is a graphic representation of the mortality rate in each group.

Toward the end of the experiment, ten pullets and ten cockerels were selected from the control group. Only chicks that had not yet shown signs of vitamin A deficiency were selected in order to show the weight curve immediately before depletion to the end point which was the death of the chick. They were weighed daily. The weights are recorded on the charts in figure 2 and figure 3. These records are of interest

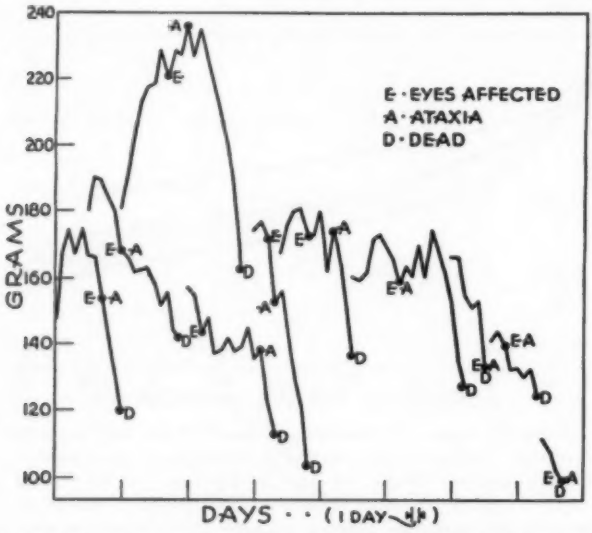

Fig. 2 Weight curves obtained from ten individual White Leghorn pullets. (Daily weighings began at twenty-eighth day from hatching.)

as indicating the type of growth curve during carotene and vitamin A depletion in chicks and the similarity of these growth curves to the growth curves of vitamin A depleted rats. Note the short length of life after the maximum weight has been reached. If chicks are to be used for quantitative tests of vitamin A after depletion, the first signs of ataxia rather than the usual 7 days of stationary or declining weight would be a safer end point to begin giving the curative dose.

For a check that an adequate amount of vitamin D was given in the form of a vitamin D concentrate produced from cod liver oil by the Zucker-Columbia University process, from which the vitamin A was oxidized, tibiae were taken from chicks toward the end of the experiment and were x-rayed. Tibiae examined and shown in plate 1 show normal structure with no evidence of rachitic lesions, thus indicating an ade-

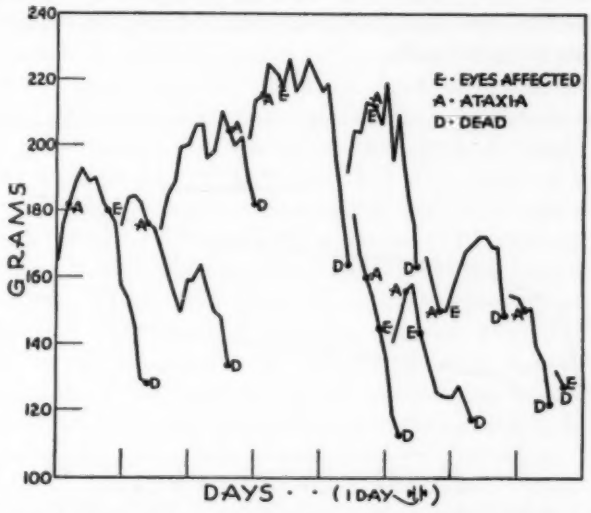

Fig. 3 Weight curves obtained from ten individual White Leghorn cockerels. (Daily weighings began at twenty-eighth day from hatching.)

quate amount of vitamin D. Not one case of 'slipped tendon' was observed in the entire group of 612 chicks. On the other hand, practically all of the 612 chicks in this experiment showed marked ataxia from 3 to 14 days before complete depletion and death. This indicates the inability of an adequate amount of vitamin D to prevent ataxia of this type in carotene and vitamin A depleted chicks. It also suggests the undesirability of using the general term of 'leg weakness' in connection with vitamin D deficiency.

Immediately following this experiment, the same solutions of carotene and vitamin A were used in another entirely unrelated experiment. This indicated a considerable loss of vitamin A in the test solutions at the end of the previous test. While this was not anticipated, it is not at all surprising in view of the fact that the chicks were given the solutions daily over a rather long period. There was considerable exposure to air during this time as the quantity of solution in the bottles decreased with a corresponding increase in the air space in each bottle.

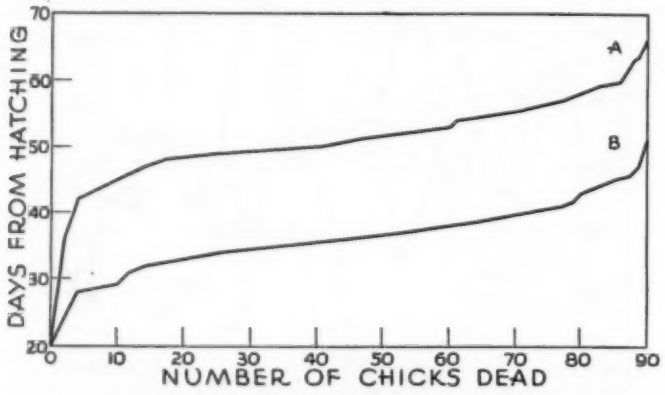

Fig. 4 Mortality curve. a) Ninety chicks each received 1000 A.D.M.A. units of carotene in oil over a period of 8 days beginning with the twentieth day from hatching. b) Ninety controls received the carotene and vitamin A-free basal diet only.

Figure 4 presents data obtained in a separate test carried out for obtaining additional information regarding the length of life of chicks on a diet free from carotene and vitamin A, and in which the possibility of loss of potency would be avoided or minimized by giving the additional vitamin A in a very short period. To study the effect of the addition of carotene in oil to the length of life, 180 day-old White Leghorn chicks were divided into two groups. a) A group of

ninety chicks each received 1000 A.D.M.A. units of carotene in refined cottonseed oil in a period of 8 days, in addition to the same basal diet used in the experiment with the 612 chicks before described. At the end of 8 days the carotene supplement was stopped and the chicks were allowed to deplete. b) A control group of ninety chicks received the carotene and vitamin A free basal diet only. Figure 4 shows the results graphically. The ninety controls lived 3315 days, an average of 36.8 days per chick, while the ninety chicks each receiving 1000 A.D.M.A. units of carotene in oil lived 4662 days, an average of 51.8 days per chick, or an addition of 15 extra days per chick for the group receiving the carotene supplement. This means that the chicks in group A lived on the average 1 extra day for each 66⅔ A.D.M.A. units of vitamin A they received as compared with the control group. Attention is directed to the apparent uniformity of storage of the two groups as evidenced by the parallel mortality curve shown in figure 4. These results are in closer agreement with our preliminary tests by the curative method (Frohring and Jersey, to be published). It will be noted in figure 4 that the addition of the carotene in oil supplement to the basal diet prolonged life and moved the mortality curve upward.

DISCUSSION

Provided that these results may be verified, this method might serve as a useful means of comparing the vitamin A content of materials of unknown potency without the necessity of having a rat colony. It is not suggested that this method would be of value in replacing the standard rat unit method, especially where the rat unit method is used for official purposes. It may be useful to laboratories that make only occasional vitamin A tests, and are unable to go to the expense of developing a rat colony suitable for the purpose.

Each time the method was used to assay an unknown, it would be necessary to run both a control group and a 'known potency' group in addition to the groups receiving materials to be tested. The 'known potency' group would be given a

standard carotene in oil solution of known potency. The extra days lived per chick and the units required to live an extra day in the 'known potency' group over that of the average days lived per chick in the control group are used as a basis for determining the approximate amount of vitamin A activity in the unknown material.

Some of the possible advantages of this method might be suggested here. Day-old chicks may be obtained at most any time of the year from recognized hatcheries. The ease of obtaining the chicks means that large numbers, say 100, may be used in each group. This would tend toward accuracy. The equipment required for chicks is comparatively inexpensive compared to that required for rats in that the chicks may be kept on brooders in groups of 100 and the individual cages necessary for rats are avoided. Since death is the end point of the method, as the animals die off the amount of work necessary diminishes rapidly. This method does not involve the weighing of the animals or the use of leg bands, as each group of 100 can be kept in separate brooder compartments.

The principal opportunity for error in this method is the separation of the chicks into groups. Due to different rations used on different breeding farms, there will be variations in the vitamin A activity stored in the egg and consequently in the day-old baby chicks received from these respective hatcheries. Bethke, Kennard and Sassaman ('27) found that eggs from hens fed cod liver oil or having access to blue grass pasture contained approximately five times as much vitamin A as eggs from hens fed the basal diet, which contained 30 per cent yellow corn, or the basal diet supplemented with alfalfa hay. Sherwood and Fraps ('32) estimated from their experimental work that 1 unit of vitamin A in the egg requires 6.3 units in the feed in addition to the maintenance requirements. They also found in four hatching tests that the percentages of eggs hatched increased with the increase in the amounts of vitamin A in the feed, but they were not related to the amounts in the eggs themselves. Either all

chicks from one breeding farm should be used or if the eggs for hatching purposes are obtained from different poultry farms, it would be advisable to divide the chicks from each farm equally into each group. A large number of animals in each group has to be depended upon to avoid the error of this variable. An interesting possibility would be the use of chicks hatched from eggs from the same hen. If uniformity could be obtained by this method, greater accuracy might be obtained with much smaller groups. Experiments along these lines are in progress.

It is interesting to note that the results obtained by this method confirm the report of Sherwood and Fraps ('32) on the relatively high vitamin A requirements of chickens as compared to rats. They say, "The maintenance requirement of White Leghorn pullets weighing about 3.2 pounds is estimated from the experiment at 105 units of vitamin A a day or about 33 units a pound. This is eight times the estimate of 4 units per day per pound for maintenance of growing rats."

Capper, McKibbin and Prentice ('31), in their work with White Wyandottes, have shown that the fowl as well as the rat can convert carotene into vitamin A, and that the vitamin A requirements of the fowl are higher than those of the rat weight for weight.

Kline, Schultze and Hart ('32) have demonstrated that chicks that have been depleted of vitamin A require more than 0.05 mg. of carotene daily in order to grow to maturity. Five-hundredths mg. of carotene would be equivalent to 100 A.D.M.A. units.

It is our opinion that the optimum requirement of the chick is much higher than that indicated by our tests. Based on the results of Green and Mellanby ('31) with the rat that four times the minimum requirement gave the optimum results as far as resistance to infection was concerned, a rather high optimum amount for chicks, probably in the neighborhood of 300 to 400 A.D.M.A. units per day is indicated. It must be borne in mind, however, that our experiments had

to do only with chicks in the age range from day-old up to about 8 to 10 weeks. The requirements for older animals may be entirely different.

The mortality curves of the control chicks fed the carotene and vitamin A-free diet, figures 1 and 4, indicate a wide variation in the amount of vitamin A activity that was carried over from the egg and that enabled the chicks to live for varying lengths of time. In figure 1 the total mortality rate of the 232 controls was 4.3 per cent for the first week (this was approximately the same in groups A, B, C and D for the first week), 6.5 per cent for the first 2 weeks, and 23.3 per cent for the first 3 weeks. By the end of the fourth and fifth weeks, these values increased rapidly to a total of 56 and 85 per cent, respectively. In other words, the total number of animals that died during the fourth and fifth weeks was 62 per cent of the entire group. All the birds were dead by the end of the seventh week. The average survival was 27.2 days. In the ninety controls in figure 4, the chicks lived from 28 to 51 days and averaged 36.8 days per chick which was about 9 days longer than the first group. This difference is assumed to be due to differences in the vitamin A activity storage of the eggs from which the chicks were hatched. The importance of setting up these tests in such a way as to permit the elimination of this variable factor is thus demonstrated. This can be done by running a parallel control group (selected from the same lot of eggs from which the experimental chicks are taken) large enough to give dependable survival figures, which are assumed to be proportional to the vitamin A activity storage of the entire lot of eggs, and which apply, therefore, also to the parallel experimental chick.

Sherwood and Fraps ('32) found that individual hens receiving no vitamin A from yellow corn lived from 34 to over 199 days and commented that it appears to be a reasonable assumption that the length of life of the pullets is a fair measure of the quantity of vitamin A stored by the pullets before the experiment was begun, when they had all the green feed they could eat. If such is the case the amount of vitamin

A stored by different pullets varied to a considerable extent and some may have contained four or five times as much as others. Sherwood and Fraps ('32) are of the opinion that these pullets fed white corn during the experiment would no doubt have died sooner had they been reared with less liberal supply of vitamin A.

Examination of the rations usually recommended and used shows that hens are frequently not supplied with sufficient amounts of carotene and vitamin A when fed without access to green feed. This probably results in many cases in low egg production, low vitamin content of the eggs, undernourishment of the body of the hen, infection and death of the fowl.

From the data here recorded it is apparent that when a ration devoid of carotene or vitamin A or both was fed to day-old chicks the onset of the reaction varied in different chicks, no doubt due to the variations in storage from the egg from which the chicks hatched which in turn was dependent upon the ration of the parent fowl. The addition of carotene or vitamin A to the carotene and vitamin A-free ration delayed the appearance of deficiency symptoms and prolonged life in a rather definite relation to the amount of carotene or vitamin A added to the diet.

SUMMARY

1. The minimum vitamin A requirement of the chick is relatively high per pound of body weight as compared to the rat.

2. The minimum requirement of vitamin A of the White Leghorn chick at the age of about 8 weeks is approximately 65 A.D.M.A. units per day.

3. Results were obtained by comparing the average number of days lived per chick in relation to the number of units of vitamin A supplement received as compared to the average of the control group.

4. Chicks offer interesting possibilities as test animals for vitamin A.

5. No cases of 'slipped tendon' were observed in 792 White Leghorn chicks depleted in vitamin A, but given a diet adequate in other respects.

6. Practically all of the 792 chicks depleted in vitamin A showed marked ataxia 3 to 14 days before complete depletion and death, even though given an adequate amount of vitamin D. This suggests the undesirability of using the broad term 'leg weakness' as related to vitamin D deficiency.

7. There is a wide variation in the number of days chicks will live on a vitamin A-free diet, no doubt due to variation in storage from the egg from which the chicks hatched, which in turn was dependent on the storage or ration of the parent fowl. The addition of carotene or vitamin A to the carotene and vitamin A-free ration delayed the appearance of deficiency symptoms and prolonged life in a rather definite relation to the amount of carotene or vitamin A added to the diet.

We are indebted to Mr. F. D. Baird, of the Nutritional Laboratory of the National Oil Products Company, Inc., for making the x-rays of the tibiae, and also to Miss Esther Zurcher for valuable assistance in checking the statistical data.

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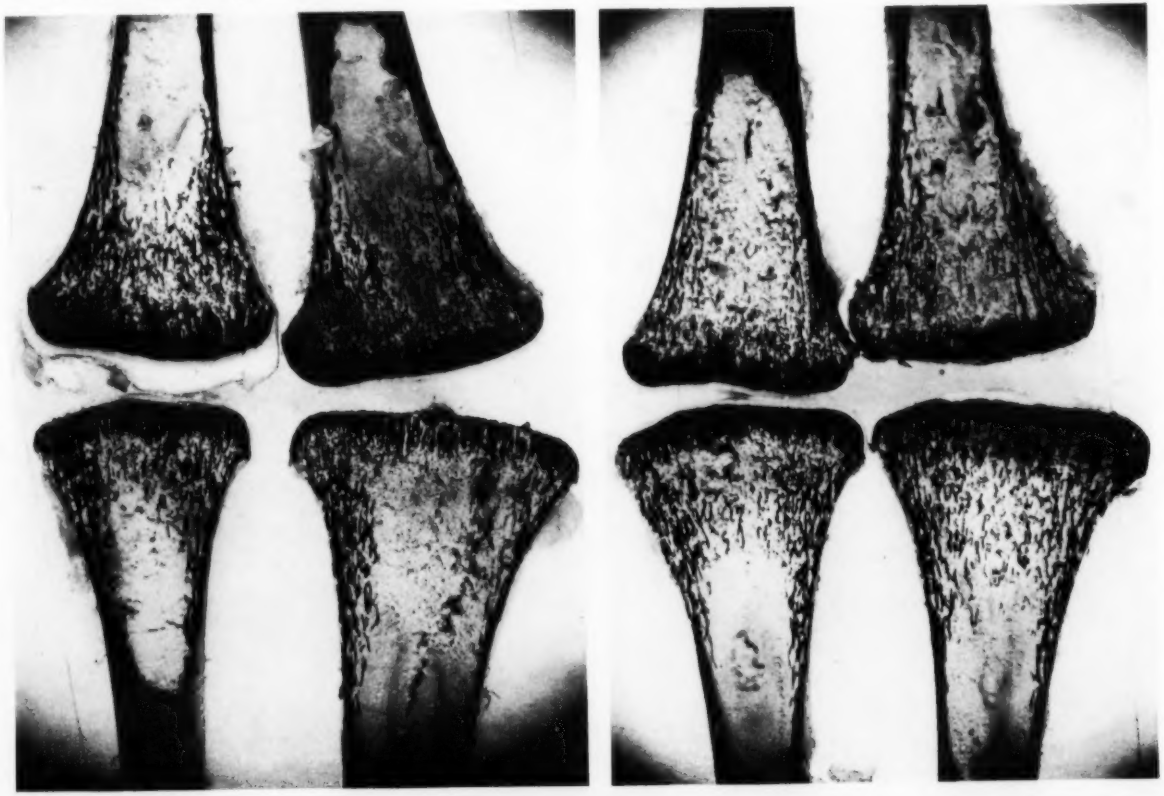

Typical x-rays of the tibiae.

THE EFFECT OF VARIATIONS IN THE DIET ON THE ABSORPTION OF FOOD IN THE ABSENCE OF PANCREATIC DIGESTION

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ONE FIGURE

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When the pancreatic juice is completely excluded from the intestine the digestion of fat and protein is much disturbed. The experimental evidence of the truth of this statement was furnished by the work of Hess ('08) and Pratt, Lamson and Marks ('09) which has since been confirmed by Brugsch ('19) and Licht and Wagner ('27). It had been known since the early observations of Abelmann (1890) that total pancreatectomy produced great loss of fat and nitrogen in the stools. After the pancreatic juice is shut off from the intestine the animals usually lose weight rapidly and usually die of inanition unless fresh pancreas is fed.

In spite of the absence of pancreas juice, however, McClure, Vincent and Pratt ('17) observed fairly good absorption of nitrogen and fat for short periods in dogs with occluded pancreatic ducts and in those completely depancreatized.

Falcon-Lesses and Herschenson in this laboratory showed that it was possible to keep dogs deprived of pancreatic juice in good condition for months with no definite loss of weight or strength without the aid of fresh pancreas by feeding definite quantities of milk powder, carbohydrates and yeast. A completely depancreatized dog receiving insulin retained its weight and strength for a year without being fed any

pancreas. These studies have been briefly reported by Pratt ('31).

The present investigation was undertaken to determine the effect of changes in the quantity and quality of the food on the absorption of nitrogen, fat and carbohydrates.

METHODS

The three dogs employed in these experiments were operated on by Dr. L. R. Whitaker. A midline incision was made and the mobile duodenum was delivered with the pancreas. A systematic search for pancreatic ducts was made from one end to the other of the corpus pancreatis. Two or three were usually encountered and these were ligated with silk thread both distally and proximally and then severed. Several small blood vessels were ligated, but the remaining blood supply was adequate for the nutrition of the duodenum. The omentum was inserted between the duodenum and the pancreas.

The dogs were kept in metabolism cages throughout the experiments. Absorption periods extended over 3 days and were demarcated by the inclusion of carmine or charcoal in the diet. Feeding took place once a day at 4 P.M. All stools were collected under alcohol and dried first over a water bath and then in a desiccator. Well mixed pulverized specimens were used for analyses and all were done in duplicate.

Nitrogen determinations were performed by the Kjeldahl method. Fats were estimated by the Saxon method ('14) and the percentage of fatty acids was expressed in terms of stearic acid.

For the analysis of the carbohydrate contents we employed an unpublished method developed by Hjort working with Pratt and later modified in this laboratory by Herschenson and Falcon-Lesses. A sample of dried stool is carefully weighed. The sample should be about 2 gm. when the carbohydrate content is suspected to be low, or 1 gm. when high. To the stool specimen in a 500 cc. beaker are added 200 cc. of distilled water and 2 cc. of concentrated hydrochloric acid

(sp.gr. 1.135). This is boiled with a reflux condenser over a sand bath for 2 hours. After cooling, 5 gm. of permutit and 5 gm. of Lloyd's alkaloidal reagent are added and the mixture shaken for 5 minutes. Then 10 per cent sodium hydroxide is added until the preparation is just acid to litmus. By means of a suction filter a clear filtrate is obtained and diluted to volume in a 1000 cc. volumetric flask. The Folin 1929 copper reduction method is carried out on 2 cc. of this filtrate. Careful washings with distilled water must be performed on the contents of the filter as well as when transferring from the beaker to the filter.

The three dogs chosen for these experiments were all females. Following the operation dog 21 and dog 22 were active and hungry on the next day, passing typical 'pancreatic stools' on the third day. Dog 20 refused to eat except for an occasional nibble of finely chopped meat for 3 weeks. She then began to eat well and to gain in weight and strength.

After recovery from the operation the dogs had voracious appetites. The urines, tested with Benedict's qualitative reagent twice a week, showed no sugar except on one or two occasions when only a very slight reduction (green with precipitate) was found. Fasting blood sugars done by the Folin-Malmros method were always below 100 mg. per cent. At no times did the dogs have polydipsia.

Each animal was put on a 3-day metabolism period as soon after the operation as possible. The dogs weighed 7.8 to 9.8 kg. before operation. The composition of the diets used is given in table 1. The daily standard diet consisted of a mixture of 50 gm. of milk powder,[1] 75 gm. of cracker meal, 50 gm. of Valentine dried extracted beef and 5 gm. of Harris brewer's yeast. By several analyses the average food value of this diet during the 3-day period was found equivalent to 245 gm. of carbohydrates, 195 gm. of protein (or 31.2 gm. of nitrogen) and 58.1 gm. of fat, or 2326 Calories. Roughly, this amounts to 95 Calories per kilogram, with 8 gm. protein per kilogram, for each dog daily.

[1] Klim.

TABLE 1

List of diets used with amounts fed daily

No. 1	Standard:	Dried milk, 50 gm.; cracker meal, 75 gm.; dried extracted beef, 50 gm.; yeast, 5 gm.
No. 2	Double standard:	Dried milk; 100 gm.; cracker meal, 150 gm.; dried extracted beef, 100 gm.; yeast, 10 gm.
No. 3	Triple standard:	Dried milk, 150 gm.; cracker meal, 150 gm.; dried extracted beef, 150 gm.; yeast, 5 gm.
No. 4	High carbohydrate A:	Dried milk, 25 gm.; cracker meal, 150 gm.; dried extracted beef, 25 gm.; yeast, 5 gm.
No. 5	High carbohydrate B:	Dried milk, 25 gm.; cracker meal, 175 gm.; dried extracted beef, 25 gm.; yeast, 5 gm.
No. 6	High nitrogen A:	Dried milk, 50 gm.; cracker meal, 25 gm.; dried extracted beef, 150 gm.; yeast, 5 gm.
No. 7	High nitrogen B:	Dried milk, 25 gm.; cracker meal, 25 gm.; dried extracted beef, 200 gm.; yeast, 5 gm.
No. 8	High fat A:	Dried milk, 50 gm.; cracker meal, 50 gm.; dried extracted beef, 50 gm.; olive oil, 30 gm.
No. 9	High fat B:	Cracker meal, 75 gm.; 'Nucoa' (cocoanut oil), 100 gm.; dried extracted beef, 50 gm.; yeast, 5 gm.
No. 10	Meat and lard:	Beef heart, 150 gm.; cracker meal, 75 gm.; lard, 20 gm.; bone ash, 10 gm.
No. 11	Large diet of meat and lard:	Beef heart, 400 gm.; cracker meal, 150 gm;. lard, 60 gm.; bone ash, 50 gm.

EXPERIMENTAL RESULTS

The figures given in table 2 on the dogs without pancreatic digestion while on the standard diet are strikingly different from those on the normal dog taking the same diet which was studied as a control. The increased weight of the feces is an outstanding feature in all the experiments on the operated dogs, the dried feces weighing three to four times as much as those passed by the normal dog. The table shows that the absorption of carbohydrates, protein and fat when compared with the normal is found to be somewhat lessened in every experiment. The absorption of starch is least affected in all and that of nitrogen most disturbed in four out of the

six test periods. The poorest absorption was that of fat, 41.5 per cent, in the experiment on dog 22.

There is no relation shown between the time that elapsed after shutting off the pancreatic juice and the efficiency of absorption. The pancreas atrophies rapidly and if the gland exerted any influence on digestion and absorption after all the ducts are occluded by the passage of the digestive ferments into the blood and thence through the bile into the intestine, as the work of Zucker, Newburger and Berg ('32) suggested, this would be expected to lessen as the pancreas

TABLE 2

Absorption studies with the standard diet on dogs deprived of pancreatic digestion

DOG	NUMBER OF EXPERIMENT	TIME AFTER OPERATION	DRY WEIGHT OF FECES IN GRAMS		ABSORPTION		
			Test period	Per day	Cho per cent	N per cent	Fat per cent
21	1	1 week	120	40	95.8	60.0	73.1
21[1]	6	7½ months	148	37	97.3	57.1	56.0
20	1	1 month	169	56.3	83.5	56.4	60.3
20	6	5½ months	114	38	97.3	55.9	86.9
20[1]	7	10½ months	204	51	95.2	47.0	67.8
22	1	2 weeks	170	56.7	92.2	56.7	41.5
Normal dog as control	1	....	37	12.3	99.0	91.2	91.3

[1] Metabolism periods carried over 4 days instead of 3 days.

shrunk. At the end of 4 or 5 months, we have found it reduced to a narrow strand not weighing more than a few grams. The pancreas of dog 21, killed 8 months after the separation of the pancreas from the duodenum, was found to be only 4 cm. long, 1 cm. wide and 2 mm. thick. Dog 20, however, showed better absorption of starch and fat 10 months after the operation than she did after 1 month; and the feces passed daily during the test weighed less. There is no evidence, however, that the stomach or intestine compensates for the lack of pancreatic digestion with the passage of time. This is shown by the studies on dog 21. She absorbed less nitrogen and fat 7 months after the operation than at the end of the first week.

Of our three dogs, dog 22 seemed to recover from the effects of the operation more quickly than the others. She was kept in a large animal room in another department and was fed 150 gm. of ground beef heart, 75 gm. of cracker meal, 10 gm. of lard and 5 gm. of bone ash each day. The dog received yeast for only 6 days. During the first month following the operation she dropped from 9 kg. in weight to 8 kg., but maintained her strength and normal state of activity. However, after another month the effects of inanition were obvious, although the unoperated dogs in the same room thrived. She became so feeble during the last 2 weeks of her life that she could barely walk, and her appetite, which was formerly voracious, became very poor. Three months after operation she was found dead. Her weight had fallen to 6.0 kg.

In contrast to dog 22, dogs 20 and 21 were kept in our own animal room and received milk powder and yeast in their diet. These animals remained in a healthy and strong condition. An attempt was made to keep the weight near the preoperative level by varying the quantity of food in the diets each week or every other week. The mechanism of maintaining their weight was very labile. When kept on the above-mentioned standard diet they tended to lose weight and showed a desire for more food.

The figure shows the weight responses during a period of a month and a half, to the standard diet and to one almost three times its size. After having been kept on a small diet for a length of time, the animals ate the large diets greedily for about a week to 10 days, when their appetites began to decrease and they rejected more than half of the food offered them. Parallel to this they gained weight at first on the large diet, but seemed better when put back on the standard diet. We carried dog 20 through three such cycles in addition to the one shown in the chart with similar results. No single optimum diet was found, but the amount of food was changed dependent on the weight and condition of the dog. In this way the animals were kept in good shape throughout the months of study.

In order to find the cause of the increased weight of the dogs when on large diets, absorption studies were carried out during those periods. It was found that large quantities of the additional food were absorbed and the general condition of the animals improved. These results are tabulated in tables 3, 4 and 5, where the food values of the various diets are given with the percentage of the food absorbed during the 3-day metabolic periods to serve as an index of the efficiency of absorption when on the various diets.

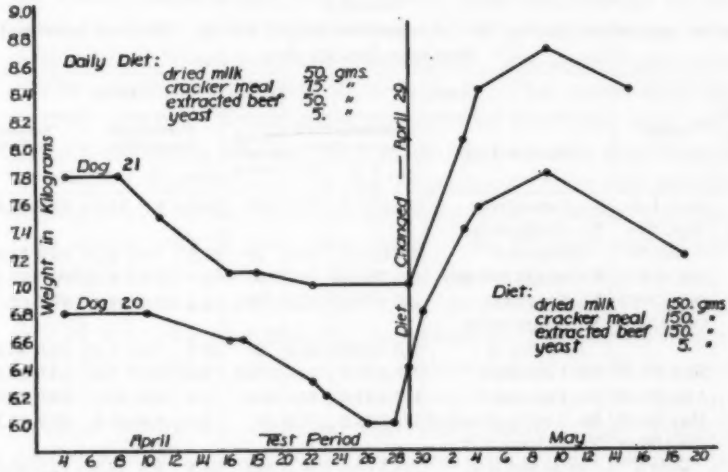

Fig. 1 The effect of an increase of diet on the weight of the dogs deprived of pancreatic external secretion.

In table 3 are summarized the results of ten absorption experiments on dog 20. These observations extended over a period of 11 months. Although no pancreas was fed, this dog without any pancreatic digestion remained in good condition after recovery from the operation. Preoperative weight was 7.8 kg. Operation July 22, 1931. Weight in June, 1933, was 7.2 kg. The dog was then in excellent condition nearly 2 years after the operation. She died February 4, 1934, of acute entero-colitis. The atrophied pancreas formed

a narrow cord 4 cm. long. The ability of this dog deprived of pancreatic digestion to live and remain in good condition for many months without the aid of raw pancreas in the diet confirms the results reported by Pratt ('31). In previous work Pratt had found that dogs deprived of pancreatic secretion when allowed to eat freely usually failed to absorb about 50 per cent of the nitrogen and fat of their food. The result was a slowly progressive loss of weight ending in death from inanition unless fresh pancreas was given. Many other ob-

TABLE 3

Absorption experiments on dog 20. Preoperative weight 7.8 kg. Pancreas separated from duodenum July 22, 1931

NUMBER OF EXPERIMENTS	DATE	DIET				FECES						
		Number and type	Food value for 3 days in grams			Dried weight, grams	Percentage composition			Percentage absorbed		
			Cho	N	F		Cho	N	F	Cho	N	F
1	Sept. 1–3	No. 1 standard	245.5	31.2	58.0	169	23.9	8	13.6	83.5	55.8	60.3
2	Sept. 29–Oct. 2	No. 4 high carbohydrates A	396.0	21.7	32.5	70	21.5	7.81	4.25	96.2	74.9	90.8
3	Oct. 5–8	No. 6 high nitrogen	125.7	68.98	84.3	161	0	12.81	4.56	100	71.5	91.3
4	Oct. 13–15	No. 8 high fat	185.4	29.95	147.3	99	4.4	11.3	9.4	97.7	62.7	93.7
5	Oct. 19–21	No. 4 high carbohydrates A	515.7	24.28	33.9	127	30.7	7.87	5.25	92.6	58.8	79.9
6	Nov. 16–18	No. 1 standard	245.4	31.2	58.0	114.5	6.25	12.05	6.65	96.9	55.8	86.9
7	Apr. 25–28	No. 1 standard	327.2	41.6	77.4	204	7.7	10.8	12.2	95.2	47.0	67.8
8	May 23–26	No. 3 triple standard	538.5	88.2	171.9	531	9.3	9.45	13.6	90.8	43.1	58.0
9	June 30 to July 2	No. 11 large diet meat and lard	358.5	59.5	256.5	592	16.8	3.8	26.4	72.2	62.6	39.6
10	July 9–11	No. 10 meat and lard	179.4	23.2	89.1	242	21.6	5.6	15.0	70.8	41.8	59.3

servers have found that dogs devoid of pancreatic secretion died within a short period of time unless given pancreas in their food. The usual diet was beef, to which sometimes lard or other fat was added. Penau and Simonnet ('26), however, kept a depancreatized dog receiving insulin, alive and in good condition for more than 2 years without feeding raw pancreas or pancreatic preparations. They attribute their success to the use of sugar in the diet and their method of feeding. The dog was given six small meals a day with an interval of 2 hours between meals. At each feeding it re-

ceived 20 gm. of beef, 10 gm. of sugar, 50 cc. of fresh milk, 20 gm. of bread, and 5 gm. of powdered bone. For an evening meal 200 gm. of meat were given. No absorption studies were made. Our dogs that lived for many months received 50 gm. or more of milk powder as a part of their regular diet and 5 gm. of yeast powder daily. They were fed only once a day.

Two special metabolism periods were included in our studies to give a basis of comparison with the results obtained by Pratt, Lamson and Marks ('09). The diet similar to that used by these workers consisted of beef heart, cracker meal and lard, to which bone ash was added. The results are given under experiments 9 and 10 in table 2. The stools were unusually heavy. When large amounts of beef and lard were fed (experiment 9) less fat was utilized by this dog than in any other test-period. In the experiment with the smaller amounts of beef and lard the percentage of nitrogen absorbed was lower than in any other test. The findings agree with the earlier work published from the Harvard laboratory.

During some metabolic periods (experiments 2, 3 and 4) dog 20 showed such excellent absorption of both carbohydrates and fats that one might have doubted whether all the pancreatic juice had been excluded from the intestine had not studies before as well as afterward shown the typically diminished absorption usually obtained in animals deprived of pancreatic juice.

The studies on dog 21 (table 4) were made for the most part simultaneously with those on dog 20 using the same diets, but the percentage of absorption was, as a rule, lower. The condition of the dogs also showed a decided contrast at the times the earlier tests were made. Dog 20, more accustomed to laboratory life, was placid, ate with good appetite, but not voraciously, usually gained and lost weight only slowly when the diets were increased or decreased, respectively, and seemed to be in the best of health. Dog 21 was decidedly unhappy with confinment to a cage; bolted all her food, showed very labile weight responses with changes in the size of the

diets, and seemed to lose considerable strength. This weakness, however, was temporary, lasting about 2 months.

From the comparative study of these 2 dogs over a period of 8 months it is clearly evident that unknown factors cause such great variation in absorption that it is very difficult to compare the value of different diets. When dog 20 was given a high carbohydrate-low fat diet the bulk of the feces was

TABLE 4

Absorption experiments on dog 21. Preoperative weight 9.8 kg. Pancreas separated from duodenum October 7, 1931

NUMBER OF EXPERIMENTS	DATE	DIET				FECES						
		Number and type	Food value for 3 days in grams			Dried weight, grams	Percentage composition			Percentage absorbed		
			Cho	N	F		Cho	N	F	Cho	N	F
1	Oct. 11–13	No. 1 standard	245.4	31.2	58.1	120	8.6	10.4	13.0	95.8	60.0	73.2
2	Oct. 19–22	No. 5 high-carbohydrate B	455.7	23.0	33.2	279	46.6	5.6	9.3	71.3	75.1	22.0
3	Nov. 13–17	No. 7 high nitrogen B	97.2	85.9	77.1	358	2.8	12.5	10.8	87.7	47.8	49.9
4	Dec. 8–10	No. 9 high fat B	188.4	24.8	264.9	190	7.4	7.8	29.8	97.1	68.5	88.7
5	Dec. 21–23	No. 3 triple standard	538.5	88.2	171.9	699	28.1	7.8	17.7	63.5	38.2	30.3
6	April 25–28	No. 1 standard	327.2	41.6	77.4	148	5.8	12.1	23.0	97.3	57.0	56.1
7	May 24	No. 3 triple standard	179.5	29.4	57.3	178	9.1	8.8	21.8	90.9	47.0	32.5
	Killed at end of 24 hours. Contents of colon added to feces											

small and the food well utilized in two experiments. Despite the very good results obtained with this diet in these two periods one cannot conclude that it is an excellent diet for a dog without pancreatic digestion, since when it was fed to dog 21 (experiment 2) there was very poor utilization. The analysis showed that no less than 46.6 per cent of the dried stool was composed of carbohydrates and the absorption of fat in this experiment, 22.0 per cent, was the lowest obtained in the entire series.

The striking feature in the study of dog 22 is the apparent relation between poor absorption and the poor condition of the animal. During the interval of 2 weeks between the operation and the first test period the dog (table 5) was fed no milk, fat or yeast. The diet consisted of beef heart, cracker meal and lard. The fact that she had greater weight of feces and poorer absorption of fat when placed on the standard diet (table 5, experiment 1) than the other two dogs was possibly due to the absence of milk and yeast from her previous diet. She was losing weight at the time of the test. In the

TABLE 5

Absorption experiments on dog 22. Preoperative weight 9.2 kg. Pancreas separated from duodenum February 9, 1932

NUMBER OF EXPERIMENTS	DATE 1932	DIET				FECES						
		Number and type	Food value for 3 days in grams			Dried weight, grams	Percentage composition			Percentage absorbed		
			Cho	N	Fat		Cho	N	Fat	Cho	N	Fat
1	Feb. 24–26	No. 1 standard	245.4	31.2	58.1	170	10.9	8.0	20.0	92.4	56.4	41.5
2	Mar. 7–9	No. 2 Double standard	490.8	62.4	116.2	405	10.9	8.9	21.3	91.0	42.3	25.7

second experiment when given twice the usual standard diet the utilization of starch, protein and fat was still less. The dog continued to fail and died apparently of inanition.

Absorption of carbohydrates. When only 126 gm. of starch were fed to dog 20 (experiment 3), and that in the form of cracker meal and dried milk, the carbohydrates were completely utilized. Dog 21, however, when given a similar diet absorbed only 87.7 per cent of the starch. When given a very large amount of starch, he showed still poorer absorption, namely, 71.3 per cent. The poorest utilization of starch in our study was 63.5 per cent. This result was obtained on dog 21 when fed three times the standard diet (experiment 5). When given the same diet later, he absorbed 90.9 per cent of the starch! The results of these two experiments show

clearly that the absorption of starch is largely influenced by factors independent of the diet.

The importance of feeding carbohydrates to dogs deprived of their pancreatic secretion has not been sufficiently recognized. Its absence from the diet may explain in part the rapid emaciation and speedy death of animals often observed after obstructing the flow of pancreatic juice into the intestine. Our dog 22, however, lost weight and finally died of inanition, although fed on an abundance of carbohydyrates. Brugsch ('19) fed his dogs, in which all pancreatic ducts had been tied, horse meat and beef suet. One lived only 3 weeks and the other 5 weeks. It should be emphasized that carbohydrates in any diet are well utilized in the absence of pancreatic digestion.

Absorption of nitrogen. On a well-balanced diet the absorption of protein is usually more disturbed in the absence of pancreatic digestion than that of fat or carbohydrates. The amount of nitrogen found in the stools of a normal dog taking 150 gm. of beef heart, 70 gm. cracker meal, 10 gm. of lard and 5 gm. of bone ash, in a 3-day experiment, was 2.1 gm., the percentage absorbed being 90.8. In an observation on another normal dog taking the standard diet the percentage of nitrogen absorbed was 91.2. When dog 20 was given exactly the same amount of food the nitrogen in the stools was 13.8 gm., the percentage absorbed dropping to 55.8 per cent. The smallest amount found in the feces in any of the studies on these dogs was 5.5 gm. The percentage absorbed was 74.9. This was on a high carbohydrate diet. The poorest nitrogen absorption was shown by dog 21 when given three times the standard diet. It was 38.2 per cent.

Absorption of fat. McClure, Vincent and Pratt ('17) found that their depancreatized dogs absorbed 17.6 to 45.3 per cent of fat when given butter or lard. Their experiments proved that, under certain conditions in the absence of the pancreas, fat may be absorbed from the intestines in relatively large amounts, ranging from 19.8 to 179.4 gm., during the experimental periods of 3 to 5 days, even when the fat fed is not

in the form of an emulsion. These experiments were made before the discovery of insulin and as a result the dogs had an unchecked fatal diabetes.

Ten experiments were made on dog 20 to determine the influence of variations in the diet on the absorption of fat. Although her pancreas was present and she did not have diabetes, in no experiment did she absorb fat as well as did the depancreatized dog in the above test.

Marked variations in absorption of fat occur independently of the diet, as is clearly shown by the results in three experiments on dog 20 when the same standard diet was given. In September 60.3 per cent of fat was utilized, in November 86.0 per cent and in the following April 67.8 per cent. The first two experiments on dog 21 likewise show that absorption cannot be predicted from knowing the food fed and they illustrate our inability to find an optimum diet. In October, when given the standard diet, 73.2 per cent was absorbed, while dog 20 absorbed only 60.3 per cent of the fat in this diet. As dog 20, a few weeks later, absorbed 90.8 per cent of the fat in the high carbohydrate diet, it seemed logical to expect that dog 21 would do as well or better. On this diet, however, she absorbed only 22 per cent of the fat! This was the poorest utilization of fat observed in our studies.

Dog 22 was in poor general condition and absorbed only 41.5 per cent of the fat on the standard diet in contrast to 60.3, 86.9 and 67.8 per cent by dog 20, and 73.2 and 56.1 per cent by dog 21 on the same diet.

It is evident that in dogs deprived of pancreatic digestion the absorption of fat is largely dependent on factors other than diet. It was clearly shown that milk fat was better absorbed than lard. This is not always the case, as one of the dogs studied by McClure, Vincent and Pratt (dog 3, experiments 3 and 4) absorbed 27.2 per cent when the fat fed was in the form of butter and 37.1 per cent when in the form of lard. The best absorption of fat observed in the present studies was on a high fat diet (table 2, experiment 4), but as an absorption of 90 per cent or more was also obtained on high

carbohydrate and high nitrogen diets it is evident that the proportion of the various food stuffs is not an important factor in determining the absorption of fat. A diet rich in carbohydrates and low in fat (table 2, experiment 2) was well utilized and 90.8 per cent of the fat absorbed. Almost exactly the same small amount of fat was given a few weeks later in a similar combination and only 79.9 per cent of the fat was absorbed.

SUPPLEMENTARY OBSERVATIONS

Practically all of the early workers on this subject neglected to include vitamins in the dogs' diets. That this factor is important can be readily seen in the published protocols of Hershey and Soskin ('31) where avitaminosis was commonly encountered in depancreatized dogs. In our experiments 5 gm. of Harris brewers' yeast were included in the diet of each dog daily. In spite of this, dog 21 showed signs of a lack of vitamins; toward the end of our experiments she developed a spasticity of her hind limbs, and began to grow progressively weaker. After increasing the vitamins in her diet, she regained her former strength, but retained a residual spasticity in her hind legs. Yeast has been shown by Nasset, Pierce and Murlin ('31) to have no effect on the amount of nitrogen excreted through the feces in depancreatized dogs. Its administration, however, was followed by a definitely increased retention of nitrogen in the body, but unaccompanied by any corresponding increase in weight. As the histological studies of Mottram, Cramer and Drew ('22) indicate that vitamins hasten the absorption of fat, it may be that the presence of yeast in the diet in our experiments is a possible contributory factor in producing the fairly good absorption of fat. However, this view is rendered improbable by the fact that Nasset, Pierce and Murlin's dogs failed to gain weight during the periods when yeast was included in the diet.

It must be emphasized that, although our experiments show that fairly good absorption is possible when all the pancreatic ducts are ligated, the stools still showed most of the character-

istics due to the absence of pancreatic juice. During our experiments we always had large bulky stools with an absolute increase in the dry weight. When the diets were large, starch granules, particles of undigested beef, and fat droplets could easily be found by the usual microscopic methods. At times these were not all present in a single stool specimen in sufficient quantities to be readily demonstrated, but at least one, and usually two of the three food constituents could be found in the feces.

The largest portion of fats in the stools (45 per cent to 60 per cent) was found split in the form of fatty acids and soaps. This is a confirmation of the results of earlier experimenters. When olive oil and cocoanut oil were fed (tables 3 and 4) a large percentage was found to be absorbed in each case.

DISCUSSION OF RESULTS

From the data presented it can be readily seen that a very large amount of carbohydrates was absorbed by the dogs when the diets were rich in starch. The absorption of nitrogen and fat was much less, both relatively and absolutely. The largest amount of nitrogen utilized was 41.5 gm., but this was only 47 per cent of the nitrogen of the diet.

The greatly increased absorption on the large diets occurred in the face of much waste and poor efficiency, as was evidenced by the fact that much more of the ingested food was found in the stools when the large diets were fed. During the same periods represented in chart 1, while on the standard diet dog 21 passed 460 gm. in 4 days, whereas on the large diet she defecated 575 gm. in 1 day. Dog 20 during the corresponding periods passed 611 gm. in 4 days in contrast to 1430 gm. in 3 days when the larger quantity of food was given. This waste is also manifested when the percentage of food absorbed is calculated. Despite the fact that the actual amount of food absorbed was larger with the larger diets, the percentage of carbohydrates, nitrogen, and fats absorbed dropped, in the case of dog 21 from 96.5 per cent to 90.9 per

cent, from 58.4 per cent to 47.0 per cent, and from 64.7 per cent to 32.5 per cent, respectively, and in the case of dog 20 from 96.2 per cent to 90.8 per cent, from 51.4 per cent to 43.1 per cent, and from 77.3 per cent to 58.0 per cent, respectively. This same behavior was observed in a similar experiment with dog 22.

CONCLUSIONS

1. Dogs with pancreatic juice wholly excluded from the intestine can be made to absorb large quantities of food, including fats, even though a high percentage of the nitrogen and fat of the diet may appear in the stools. The carbohydrates fed are usually well absorbed in the absence of pancreatic digestion.

2. These dogs can live for long periods of time in a relatively healthy condition without the addition of fresh pancreas to the diet.

3. They can be made to gain weight and absorb increased amounts of food when their diet is increased, but this is done with impaired efficiency, the animals losing more in their stools. Also, the dogs cannot tolerate these large diets over a long period of time.

4. The improved absorption is not related to variations in the size of the carbohydrate, protein, or fat fractions of the diet.

5. Not only do different dogs deprived of pancreatic digestion absorb different amounts of starch, nitrogen and fat on the same diet, but the same dog on the same diet at different times may absorb very different amounts. From these findings it is evident that unknown factors influenced profoundly the absorption of food in our dogs.

6. The better absorption and the better general health of our dogs compared with those reported by most workers seem due chiefly to the standard diet we employed which contained a liberal amount of carbohydrates, milk fat and vitamins.

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